ENDOCRINOLOGY

VOLUME 47 NOVEMBER, 1950 NUMBER 5

STUDIES ON THE METABOLISM OF RADIO-PROGESTERONE IN MICE AND RATS*

BYRON RIEGEL, W. L. HARTOP, JR. AND G. W. KITTINGER

From the Chemical Laboratory, Northwestern University, Evanston, Illinois

THE synthesis of progesterone-21-C^{14} (Riegel and Prout 1948) has made possible the investigation of certain specific problems involving the metabolism of the hormone. The presence of C^{14} at the 21 position serves the dual purpose of labeling the hormone as well as providing a substance suitable for study of the metabolism of the side chain.

An important objective of this investigation was to determine whether animals have the ability to oxidatively metabolize the side chain of progesterone. Radioactive carbon dioxide was detected in the expired air of rats and mice after the injection of progesterone-21-C^{14}. This metabolic pathway for the oxidation and elimination of the C-21 carbon atom of progesterone is, therefore, established.

A second important objective of this investigation was to determine the distribution of progesterone or its metabolites within the tissues of the animal organism. Various tissues of rats and mice have been shown to possess different capacities for the absorption and retention from the body fluids of progesterone or its metabolites.

EXPERIMENTAL PART

Procedures were required which would allow the quantitative determination of radioactivity in the expired air, feces, urine, and various tissues of the animal. Well-established methods were used whenever possible. If no suitable procedures existed, methods were developed and tested in preliminary determinations with known quantities of the radioactive hormone. The scheme described for the assay of the radioactive content of the tissues was developed from the experience gained in several pilot determinations. These initial investigations were carried out on mice in order to conserve the limited supply of radioprogesterone. The results reported in this paper were obtained after the experimental procedures had been standardized.

Mature female mice weighing approximately 25 gm., and rats weighing approximately

Received for publication March 23, 1950.

* This work was supported by a Grant-in-Aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council and by a grant from Swift and Company.

250 gm. were used. The animals, except where indicated, were obtained from commercial inbred stock colonies.

The physiological state of the animals was determined prior to the administration of the hormone. Studies were made using animals in various stages of estrous, pregnancy, and pseudopregnancy. The estrous stages were determined by the vaginal smear technique of Allen and Doisy (1924). Pseudopregnancy was induced by mechanical stimulation of the cervices of animals in heat.

A standard solution containing 0.211 mg. of pure crystalline progesterone per 0.25 cc. of sesame oil was used for injection. The specific radioactivity of the pure hormone was 1500 counts per minute (c.p.m.) per μg. Injections were, except for one case, intraperitoneal. Dosages were varied so as to correspond to values from 80 μg. to 0.211 mg. of progesterone. Higher than physiological dosages were used in most cases to enhance the elimination of the C-21 carbon. The elapsed time between injection and sacrifice (using ether) was varied from two and one-half to forty-eight hours to determine the effect of time on absorption and excretion.

A slight modification of the apparatus described by Skipper and Bryan (1948) for metabolic studies was used to obtain the carbon dioxide from expired air. The animals on which respiratory studies were made were placed in the metabolism cage immediately following the injection. Carbon dioxide-free air was drawn through the apparatus at a rate of 200–300 cc. per minute. The carbon dioxide in the expired gases was absorbed in 10 per cent sodium hydroxide solution as described by Skipper and Bryan (1948). A stopcock arrangement permitted the uninterrupted exchange of receivers so that samples could be collected at any desired interval.

The alkaline carbonate solutions in the gas washing bottles were quantitatively transferred to Erlenmeyer flasks. The solutions were warmed on a steam bath and the carbonate precipitated with barium chloride. The barium carbonate was filtered into tared sintered glass funnels. After thoroughly washing with distilled water and acetone, the precipitate was dried and weighed.

The total feces obtained at the end of the run were removed from the metabolism chamber and combined with the contents of the intestinal tract. This material when dried *in vacuo* over phosphorus pentoxide for three days provided a product which could be ground to a fine powder. An aliquot portion was oxidized by the wet combustion method of Van Slyke and Folch (1940) in an apparatus which was a simplification of that used by Skipper and Bryan (1948) for tissue oxidation. The carbon dioxide resulting from this oxidation was converted to barium carbonate in the manner already described.

One of the methods given by Dauben, Reid and Yankwich (1947) was used to prepare the samples of radioactive barium carbonate for counting. This procedure was the evaporation of suspensions of barium carbonate in ethanol on aluminum discs. The aliquot of barium carbonate was obtained from the difference in weight of the disc before and after deposition and the total weight of the original precipitate of barium carbonate.

The radioactivity of the discs was measured with the Radiation Counter Laboratory's Nucleometer, Mark 9, Model 1, and Scaler, Mark 13, Model 1, with the counting tube shielded with lead bricks. A calibration curve for the correction of self-absorption was prepared from a standard sample of radioactive barium carbonate by the procedure reported by Yankwich (1947). All corrections for self-absorption and background are included in the experimental data.

A summary of the results obtained in the respiratory studies is given in Table 1. The dosage was 0.211 mg. of radioprogesterone (316,000 c.p.m.) in sesame oil injected intraperitoneally. Run M-2 was extended to forty-eight hours to attempt maximum recovery of expired radiocarbon.

The fecal material from run M-2 contained radioactivity equivalent to 51.5 per cent of the total injected radiocarbon. Extrapolation of the data obtained in the respiratory studies of run M-2 accounted for an additional 26 per cent.

The total recovery of radioactivity from these two sources was 77.5 per cent.

Run D was made with a 280 gm. female white rat in proestrus. The animal was injected with 0.186 mg. of radioactive progesterone and sacrificed twelve hours after injection. In the period between injection and sacrifice the rat excreted 4.7 per cent of the radiocarbon as carbon dioxide. The organs and blood of the thoracic and peritoneal cavities were pooled and an analysis made of the radioactive content. These tissues contained 5 per cent of the injected radioactivity. The feces and the gastro-intestinal tract (from the duodenum to and including the colon) were found to contain an additional 88 per cent. The total recovery from these three sources was 98 per cent.

TABLE 1. RECOVERY OF RADIOCARBON AS $C^{14}O_2$ IN THE EXPIRED AIR

Hours of collection	Run M White mouse one week pregnant		Run M-1 White mouse two weeks pregnant		Run M-2 C-57 Black mouse in estrus		Run B White rat in estrus		Run D White rat in proestrus‡	
	cpm*	% C^{14} rec.	cpm	% C^{14} rec.	cpm	% C^{14} rec.	cpm	% C^{14} rec.	cpm	% C^{14} rec.
0–1	2,650	0.85	3,560	1.12	11,500	3.63	3,090	0.98	1,800	0.64
1–2	2,970	0.94	5,310	1.68	14,900	4.70	4,330	1.36	1,900	0.68
2–3	3,120	0.99	6,900	2.18	9,300	2.93	3,160	1.00	2,340	0.84
3–4	2,790	0.88	6,790	2.14	7,450	2.35	2,330	0.73	1,820	0.65
4–5	2,290	0.72	3,470	1.09	4,930	1.55	1,330	0.42	1,540	0.55
5–6	2,410	0.76	3,520	1.11	4,450	1.40	1,130	0.36	1,560	0.56
9–10	—	—	—	—	2,230	0.70	1,755†	0.56†	2,100†	0.75†
24–25	—	—	—	—	376	0.12	—	—	—	—
31–32	—	—	—	—	313	0.10	—	—	—	—
47–48	—	—	—	—	143	0.05	—	—	—	—
6 hour totals	16,230	5.1	29,550	9.3	52,530	16.6	15,370	4.8	13,060	3.9

* Counts per minute.
† Hours of collection 6–8.
‡ Dosage 0.186 mg.

The radioactive content of the tissues at the time of sacrifice served to indicate the distribution of the progesterone or its closely related metabolic products. Since direct measurement of the radioactive content of the tissues was not feasible, the measurements were made on tissue extracts. The preparation of these tissue extracts was advantageous for many reasons. Separation of the unsaponifiable components of the tissue from protein, fats, etc., gave preparations more concentrated in radioactivity. In addition, radioactivity due to substances other than the steroids was excluded. The extracts could be evaporated so that the counting could be carried out directly on thin films of the residue. As an alternative the residue from the extracts could be oxidized to carbon dioxide.

After sacrifice, the desired tissues were removed by dissection and immediately weighed. The tissues were then hydrolyzed for one hour in 10% potassium hydroxide in alcohol. After hydrolysis, the tissues were extracted with ether in a continuous micro liquid-liquid extractor for two hours. The washed ether extract was evaporated in an amalgamated copper counting dish so that the residue was deposited as a thin uniform film of constant area. The counting was carried out in the same manner as with the barium carbonate preparations. However, corrections for self-absorption were not made.

Experiments with tissue and known amounts of radioactive progesterone were conducted as a check on the above procedure. Recovery of the radioactivity originally present was 60–65 per cent. The incomplete recovery was found to be due to the inability of the ether to remove unknown acidic transformation products from the alkaline hydrolysate. This loss of activity was constant within the limits of counting accuracy. Only

the relative differences in concentrations between tissues are considered significant. This method of treatment will not separate acidic transformation products which might be present in the tissues at the time of sacrifice of the animals. The radioactive concentrations reported in the mouse data represent only neutral unsaponifiable substances. However, by using a larger experimental animal, it was possible to refine the procedure

TABLE 2

Assay N.—White mouse, one week pregnant, sacrificed two and one-half hours after injection of 0.1 ml. (80μg.).

Tissue	Wet weight, gm.	cpm/gm.	Activity index
Pituitary	0.022	1,500	++++
Adrenals	0.007	400	++
Ovaries	0.022	400	++
Uterus	0.112	340	+
Liver	0.223	130	+
Spleen	0.095	90	+
Kidney	0.320	60	+

Assay D.—White mouse in diestrus, sacrificed eight hours after injection of a dosage of 0.25 ml.

Tissue	Wet weight, gm.	cpm/gm.	Activity index
Pituitary	0.007	1,000	++++
Adrenals	0.013	850	++++
Ovaries	0.017	590	+++
Uterus	0.069	100	+
Liver	0.564	83	+
Spleen	0.130	70	+
Kidney	0.438	37	+

Assay E.—White mouse in estrus, sacrificed eight hours after injection of 0.25 ml.

Tissue	Wet weight, gm.	cpm/gm.	Activity index
Pituitary	0.010	700	++++
Adrenals	0.012	500	++++
Ovaries	0.033	100	+
Uterus	0.105	90	+
Liver	0.624	30	+
Spleen	0.130	85	+
Kidney	0.466	30	+

Assay F.—White mouse, in estrus, sacrificed eight hours after injection of 0.25 ml.

Tissue	Wet weight, gm.	cpm/gm.	Activity index
Pituitary	0.010	5,300	++++
Adrenals	0.011	5,400	++++
Ovaries	0.041	1,400	++
Uterus	0.131	400	+
Liver	0.705	250	+
Spleen	0.155	400	+
Kidney	0.547	140	+

Assay M.—White mouse, one week pregnant, sacrificed twelve hours after injection of 0.25 ml.

Tissue	Wet weight, gm.	cpm/gm.	Activity index
Pituitary	0.013	lost	—
Adrenals	0.016	400	++++
Ovaries	0.024	0	—
Uterus	0.118	100	++
Liver	0.671	94	++
Spleen	0.112	40	+
Kidney	0.400	65	+

TABLE 2—(*continued*)

Assay H.—White mouse, in proestrus, sacrificed twenty-four hours after injection of 0.25 ml.

Tissue	Wet weight, gm.	cpm/gm.	Activity index
Pituitary	0.011	1,500	++++
Adrenals	0.010	1,400	++++
Ovaries	0.020	200	+
Liver	0.858	35	+
Spleen	0.147	14	+
Kidney	0.532	30	+

Assay J.—White mouse, in estrus, sacrificed twenty-four hours after injection of 0.25 ml.

Tissue	Wet weight, gm.	cpm/gm.	Activity index
Pituitary	0.014	400	+++
Adrenals	0.011	700	++++
Ovaries	0.024	400	+++
Uterus	0.088	10	+
Liver	0.355	190	++
Spleen	0.252	40	+
Kidney	0.497	100	+

Assay L.—White mouse, in proestrus, sacrificed twenty-four hours after injection of 0.25 ml.

Tissue	Wet weight, gm.	cpm/gm.	Activity index
Pituitary	0.010	300	++++
Adrenals	0.009	400	++++
Ovaries	0.013	80	+
Uterus	0.088	60	+
Liver	0.329	140	++
Spleen	0.084	100	++
Kidney	0.337	30	+

Run B.—White rat, in estrus, sacrificed eight hours after injection intraperitoneally of 0.25 ml.

Tissue	Dry weight, gm.	cpm/gm.	Activity index
Pituitary	0.022	1,500	+++
Adrenals	0.020	lost	—
Ovaries	0.016	1,800	+++
Uterus	0.054	410	+
Liver	2.398	2,590	++++
Spleen	0.120	800	+
Kidney	0.385	630	+
Blood	0.279 (1.3 ml.)	86	+

Run C.—White rat, three days pseudopregnant, sacrificed twenty-four hours after injection of 0.25 ml. subcutaneously.

Tissue	Dry weight, gm.	cpm/gm.	Activity index
Pituitary*	0.004	11,000	++++
Adrenals	0.008	—	—
Ovaries	0.008	3,000	++
Uterus	0.100	1,240	+
Liver	2.916	357	+
Spleen	0.123	—	—
Kidney	0.483	—	—

* Anterior lobe only.

so as to overcome this difficulty. The results from the investigation using rats clearly demonstrate that no detectable radioactive acidic products were obtained.

The data on the distribution of radioactivity in the tissues of mice are summarized in Table 2. It became increasingly apparent during the course of this work that it was

extremely difficult to duplicate absolute levels of radioactivity in the tissues in spite of the efforts to hold experimental variables to a minimum. However, the radioactivity remaining in the tissues at the time of sacrifice was distributed among the organs in a definite pattern. The data, therefore, were tabulated in a form which would stress the wide differences in concentration of radioactivity found among the tissues of every animal. An arbitrary division of the organs into four ranges of radioactive concentration readily brought out these differences in a form which was free from the effects of unavoidably large counting errors. This division is expressed in the column headed activity index. The concentrations of radioactivity found in the tissues are expressed in one to four + signs which indicate the relative degree of concentration rather than any specific concentration. Corrections for background are included in the data. All organs were used *in toto* excepting the liver, where a representative portion was chosen.

Similar distribution studies were made with rats. The tissues were removed and dried *in vacuo* over phosphorus pentoxide until no further loss of weight occurred. The dried tissues were weighed and then broken up to facilitate the extraction with boiling toluene for one hour. The toluene extract was then boiled under reflux for one hour with an equal volume of a 20% aqueous solution of potassium carbonate. This two-phase saponification is an excellent way to avoid the partial destruction of progesterone, if present, and to remove saponifiable materials from the organic layer. The toluene layer was then separated and washed with water. The washed extract was evaporated and the residue oxidized in the same manner as previously described for fecal material. The barium carbonate was recovered, plated out, and counted. Recovery experiments run as a check on the above procedure resulted in quantitative recovery of the radioactivity by conversion to barium carbonate.

No radioactive acidic products were found in the aqueous phase of the two-phase saponification of tissues or in the radioactivity recovered from the feces. All radioactivity was found in the neutral unsaponifiable fraction.

The possibility of carbon dioxide fixation was considered. If this took place, water soluble, radioactive substances would be formed. None was detected in the aqueous phase. This could be due to an overwhelming dilution from the bicarbonate pool. However, the data of many workers have shown that carbon dioxide and bicarbonate are not utilized by the rat in the synthesis of unsaponifiable materials.

The radioactive metabolites found in the feces of rats after injection of progesterone-21-C^{14} have been partially characterized by chemical methods. The method of Pincus and Pearlman (1941) for the microseparation of the ketonic, nonketonic, alcoholic and nonalcoholic steroids was used. The results of these separations demonstrate that the radioactive metabolites are ketonic and nonalcoholic. By the use of carrier technique, it was shown that progesterone was not present.

Typical data are presented in Table 2. Corrections for self-absorption and background are included in the rat data.

DISCUSSION

The injection of a labeled hormone provides a method for the quantitative study of the distribution of the hormone and its metabolites and their elimination. By considering the data from many animals, an accounting of essentially all of the injected radioactivity may be made. Mice are known to excrete steroids in the feces and not in the urine. It is not surprising that over 50 per cent of the activity was found in the fecal material. A

detailed study showed that a mouse excreted at least 26 per cent of the activity in the expired carbon dioxide. The small amount of remaining activity is distributed among the body tissues and fluids.

A general survey of the distribution of the radioactivity was made on most of the organs involved in the endocrinology of the hormone. This work confirms the results of other workers who accumulated excellent data without the advantages of a tracer.

Injection of the hormone into the environment of the peritoneum provides a large and heavily vascularized surface for the rapid absorption of the hormone. During absorption blood levels of the hormone approaching saturation might reasonably be expected. The maximum rates of respiratory excretion of $C^{14}O_2$ were invariably before the third hour after injection. Hooker and Forbes (1947) determined the solubility of progesterone in blood and found that a saturated solution contained 5.5–8 μg. per cc. At the end of eight hours, we found, however, the titre of the hormone was only 10^{-2} μg. per cc. in the blood of a rat. This fact combined with the respiratory data indicates that the concentration of the progesterone was approaching physiological equilibrium at the time of sacrifice of the animals.

The low level found in the uterus was unexpected. It was assumed that the effects of progesterone in quieting the uterine motility and in alteration of the endometrium would be accompanied by the absorption of the radioactive hormone.

The concentration of radioactivity found in the ovaries varied considerably with the physiological state of the animal. Relative concentrations were found to be low when mice were injected at estrus and sacrificed at eight hours, or injected at proestrus and sacrificed at twenty-four hours. The conditions at the time of sacrifice in both cases were presumably favorable for the synthesis of the hormone by the ovaries. A similar condition existed with the pregnant animals. In these instances the endocrine equilibria is not likely to be favorable for the absorption and storage of a hormone in the ovaries. On the other hand, if the mice were injected at diestrus and sacrificed at eight hours, or at estrus and sacrificed at twenty-four hours, the concentrations of radioactivity were found to be significantly higher. The higher absorption is observed in the physiological states in which the blood titres of natural progesterone are normally low. To establish these trends would require larger groups of animals. This, in turn, would require a more abundant supply of radioprogesterone.

The occurrence of consistently high relative concentrations in the pituitary is extremely interesting in view of the role of this organ in controlling the elaboration of progesterone by the ovaries. The data suggest that the hormone or a metabolic variation is absorbed by the pituitary as an inte-

gral part of the regulatory processes of its own elaboration. The relative concentrations of radioactivity found in this organ were remarkably independent of variations in experimental conditions.

The adrenals of mice were found to contain relatively large amounts of radioactivity. This is especially interesting in view of the knowledge that progesterone and other steroidal hormones have been isolated from these glands. The significance of this data is difficult to evaluate. Several explanations are readily apparent, one of which is that progesterone could be used in the synthesis of adrenal hormones (Hechter, 1949).

The greatest amounts of tissue radioactivity were isolated from the liver. Since this activity is distributed through a large organ, the concentrations per gram are low and misleading. The important role of the liver in the inactivation and excretion of the steroid hormones has been strongly indicated by the excellent work of others (Ferin, 1946; Cantarow, 1943; Engel, 1944; Selye, 1941). The large quantities of radioactivity found in the liver lends support to their evidence.

The concentrations found in the spleen and kidney were low. As far as is known, the spleen is not involved in the endocrinology, or the metabolism of progesterone. The kidneys, on the other hand, may well be involved in the rat.

The results of this work establish that progesterone and its C-21 conversion products are not stored in appreciable quantities in the tissues and fluids of the rat and mouse. All investigators have failed to find high concentrations of progesterone in the tissues of animals excreting relatively large quantities of progestational substances (Fieser, 1949). The metabolism, of progesterone following synthesis, and excretion are, therefore, continuous and rapid. The ability of the animal to excrete excess progesterone by the usual metabolic pathways is well demonstrated by our data. Variation of the physiological state of the animal, manner and amount of dosage, and the time of sacrifice after injection failed to produce any large changes in the amount of radioactivity retained, or in the manner of its distribution between the various organs.

Errors in the determination of the radioactivity present in individual samples of the expired air and fecal material never exceeded 12 per cent of the reported value. The errors were frequently much lower in magnitude. However, errors in the determination of the radioactive content of the tissues were usually significantly higher. This was unavoidable due to the necessity of counting at levels close to background values. This difficulty was overcome to some extent by extending the counting time on weakly radioactive samples. Counting time was prolonged on samples counting below ten counts per minute above background until the expected statistical deviation was $\pm$ one to two counts per minute. Several such counts

were made on each sample. The average of these values is reported. The samples more concentrated in radioactivity were subject to proportionately lower errors. The arbitrary groups of the activity index are sufficiently broad to overcome the effect of errors on the significance of the data.

SUMMARY

Metabolic studies related to the endocrinology of progesterone in mice and rats have been made with a labeled hormone. The hormone was not stored but rapidly metabolized and excreted. The methyl group in the side chain of progesterone-21-C^{14} was shown to be oxidatively excreted as $C^{14}O_2$ in small amounts. However, most of the radioactivity was found in the neutral unsaponifiable fraction of the feces. The distribution of radioactivity in the tissues was found, in some cases, to vary significantly with changes in the estrous and pregnancy states of the animal. The pituitary and adrenals of mice invariably contained relatively large concentrations of the injected radiocarbon. The highest concentration was observed in the pituitary of rats. The adrenals of rats contained no detectable radiocarbon.

REFERENCES

ALLEN, E., FRANCIS, B. F., ROBERTSON, L. L., COLGATE, C. E., JOHNSON, C. G., DOISY, E. A., KAUNTZ, W. B., AND GIBSON, H. V.: *Am. J. Anat.* **34:** 133. 1924.

CANTAROW, A., PASCHKIS, K. E., RAKOFF, A. E., AND HANSON, L. P.: *Endocrinology* **33:** 309. 1943.

DAUBEN, W. G., REID, J. C., AND YANKWICH, P. E.: *Indust. & Engin. Chem., Anal. Ed.* **19:** 828. 1947.

ENGEL, P.: *Endocrinology* **35:** 70. 1944.

FERIN, J.: *Compt. Rend. Soc. de Biol.* **140:** 800. 1946.

FIESER, L. F., AND FIESER, M.: *Natural Products Related to Phenanthrene*, 3rd Ed., Reinhold Publishing Corp., New York, 1949. p. 383.

HECHTER, O., JACOBSEN, R. P., JEANLOZ, R., LEVY, H., MARSHALL, C. W., PINCUS, G., AND SCHENKER, V.: *J. Am. Chem. Soc.* **71:** 3261. 1949.

HOOKER, C. W., AND FORBES, T. R., *Endocrinology* **41:** 158. 1947.

PINCUS, G., AND PEARLMAN, W. H., *Endocrinology* **29:** 413. 1941.

RIEGEL, B., AND PROUT, F. S., *J. Org. Chem.* **13:** 933. 1948.

SELYE, H., *J. Pharmocol. & Exper. Therap.* **71:** 236. 1941.

SKIPPER, H. E., BRYAN, C. E., WHITE, L., JR., AND HUCHISON, O. S., *J. Biol. Chem.* **173:** 371. 1948.

VAN SLYKE, D. D., AND FOLCH, J., *J. Biol. Chem.* **136:** 509. 1940.

YANKWICH, P. E., NORRIS, T. H., AND HUSTON, J. L., *Indust & Engin. Chem., Anal. Ed.* **19:** 439. 1947.

THE GRADATION OF EFFECTIVENESS AND ABSORPTION OF DESOXYCORTICOSTERONE ACETATE PELLETS BY DILUTION WITH CHOLESTEROL[1,2]

ALBERT SEGALOFF, M.D.

From the Department of Medicine, Tulane University of Louisiana, School of Medicine, and the Alton Ochsner Medical Foundation, New Orleans, Louisiana

SINCE their introduction pellets of steroid hormones have enjoyed wide popularity, both in experimental and clinical medicine (Segaloff 1950). The usefulness of this method was greatly extended by Shimkin's suggestion that pellets diluted with cholesterol are absorbed at a slower rate. There has been one experiment of which I am aware in which absorption of the cholesterol diluent was assessed by direct measurement of the cholesterol (Fuenzalida, 1944), and another in which the active steroid was measured by biologic assay (Shimkin, 1941). However, I know of no study in which an attempt was made to correlate the possible gradations of both effectiveness and absorption of the active steroid when progressive dilutions with cholesterol were employed. This report represents our first attempt to correlate effectiveness and absorption in pellets diluted with progressively larger amounts of cholesterol.

MATERIALS AND METHODS

Two types of desoxycorticosterone acetate (hereafter called DCA) pellets were employed. All pellets were of cylindrical shape and uniform size, weighing as close to 10 mg. as was feasible with such large numbers as were necessary for this study. The pellets were prepared either by compression in a die or by drawing up the molten material into stainless steel tubes of uniform diameter, extruding them, and subsequently cutting them with a hot wire.

After removal the pellets were dried, weighed, and dissolved in ethyl alcohol; the amount of DCA was then calculated from the absorption of light at 240 mμ.

Young male rats of the inbred Fischer strain, weighing from 40 to 60 gm. were employed in all the studies. The animals were all hopper fed Purina Laboratory Chow and tap water ad libitum.

Under ether anesthesia bilateral adrenalectomy was performed in a single sitting through one cutaneous incision. The subcutaneous pellets were implanted at the time

Received April 8, 1950.

[1] We would like to thank Mr. Jack Cooper of Ciba Pharmaceutical Products, Inc. for preparation of the pellets used in this study, and Miss Verda Powell also of Ciba Pharmaceutical Products, Inc. for performing the determinations of residual desoxycorticosterone acetate in the pellets.

[2] This study was supported by grants from Mr. F. W. Fischer and Mr. Ben May.

of operation through the same incision. The cutaneous incisions were closed as usual with wire staples.

Three types of controls were employed: animals subjected only to adrenalectomy, animals adrenalectomized followed by implantation of pellets of 100 per cent cholesterol,

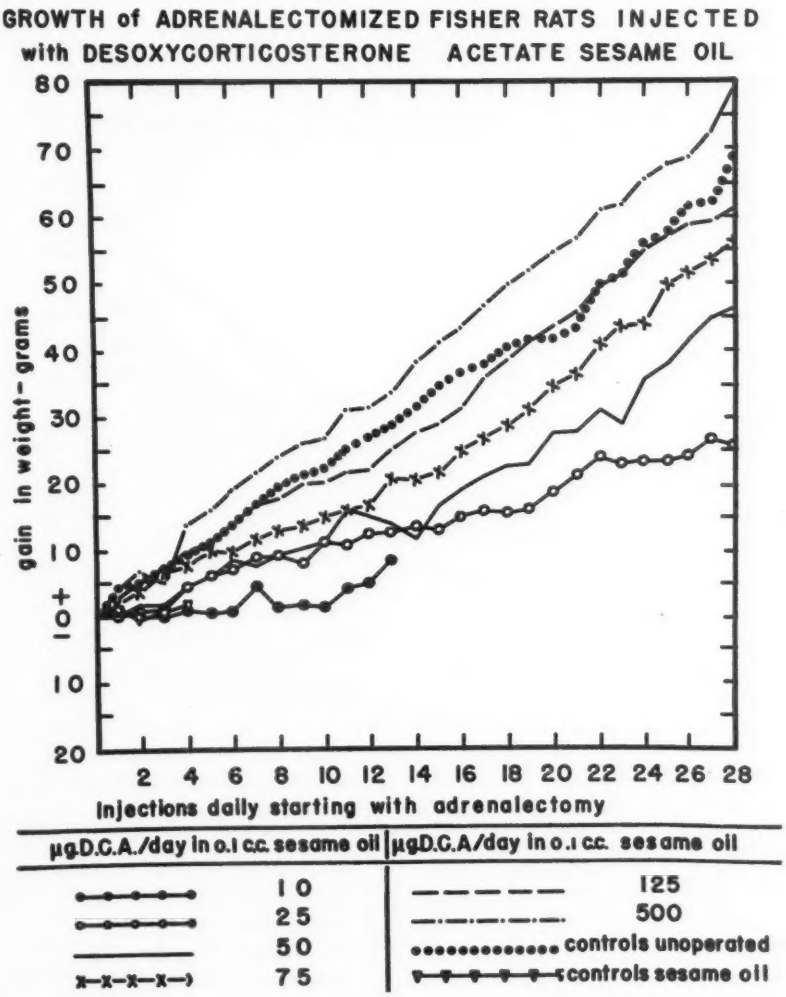


FIG. 1

and animals adrenalectomized and given daily injections of 0.1 mil. sesame oil. As a reference standard for growth curves, groups of adrenalectomized animals were given daily injections of various amounts of DCA in sesame oil. At least 20 animals were employed in each group. All animals receiving no DCA in any form died on the fourth,

fifth or sixth day following adrenalectomy. The animals were weighed daily. Treatment with the oil solutions was discontinued or the pellets were removed on the twenty-eighth day. The small number of animals which survived following withdrawal of therapy was not included in computation of the average results, since accessory adrenal tissue was observed at necropsy in all of them.

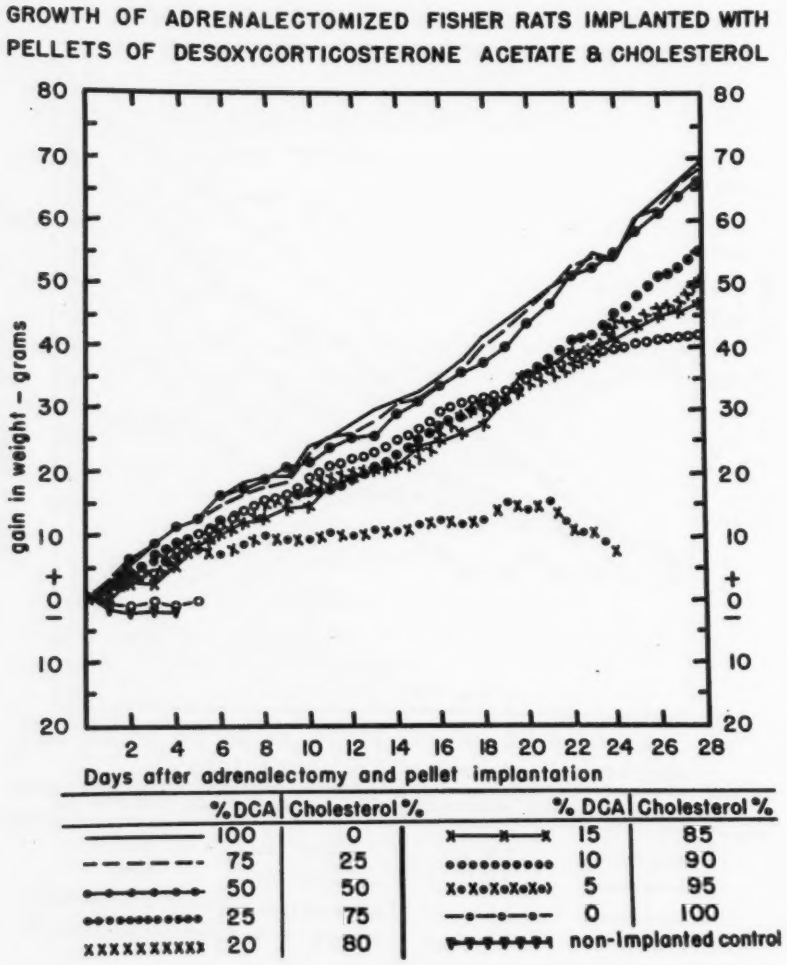


FIG. 2

RESULTS

The growth curves for the animals are presented as the average gain in weight for each group. Figure 1 shows the results obtained with injections

of DCA in sesame oil. All the sesame-oil-injected controls were dead on the fourth day. Ten μg. DCA a day per animal was insufficient for either growth or survival and none of the 20 animals survived beyond the thirteenth day. On 25 μg. DCA a day, the 70 per cent of animals that survived

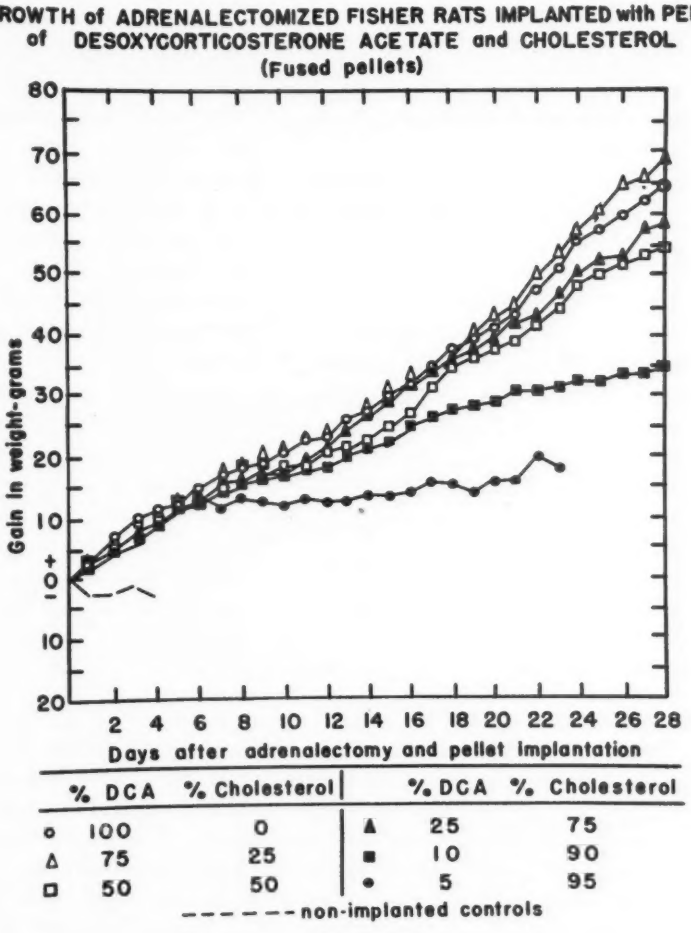


FIG. 3

the 28-day experimental period had an average gain in weight of 25 gm. All animals given 50 μg. DCA or more daily survived the 28-day experimental period and showed increasingly greater increments of growth.

Figure 2 shows the results obtained with implantation of a single, compressed pellet of DCA in each animal. All controls in which pellets were

not implanted as well as those receiving pellets of 100 per cent cholesterol were dead by the fifth day. Pellets containing 5 per cent of DCA did not maintain any of the animals beyond the twenty-fourth day. Twenty per cent of the animals receiving 10 per cent pellets survived the 28-day experimental period. These animals showed an average gain of 41 gm. in weight. Pellets containing progressively more DCA produced progressively better growth until pellets containing 50 per cent apparently yielded optimal growth which approximates that obtained in normal intact animals. No additional increment of growth was observed from use of pellets containing 75 or 100 per cent DCA.

The results obtained from fused pellets are presented in figure 3. These results are essentially the same as those obtained with compressed pellets.

Table 1 contains the assays for residual DCA content on removal of the pellets after 28 days. It is immediately apparent, that a gradation in absorption as well as growth response was obtained. Assays were not performed on all 20 pellets in each group, but a representative group of three or four was selected for each of the groups of animals.

TABLE 1. ABSORPTION OF CHOLESTEROL-DESOXYCORTICOSTERONE ACETATE PELLETS

% D.C.A.	Mg. absorbed—28 days			
	Compressed	No. pellets analyzed	Fused	No. pellets analyzed
100	7.02 mg.	3	4.28	4
75	4.30	5	4.04	4
50	2.92	3	1.42	4
25	0.91	6	1.90	4
10	0.81	4	0.74	4
5	0.31*	7	0.46*	4

* Extrapolated to 28 days.

DISCUSSION

This experiment, in which both compressed and fused pellets of DCA and cholesterol in varying percentages were employed, demonstrates that gradation in growth response in adrenalectomized animals can be obtained. It also shows that gradation in the rate of absorption is obtained which is roughly proportional to the percentage of DCA in the pellets. It is difficult to explain why less DCA was absorbed from the 50 per cent fused pellets than from the 25 per cent pellets but it should be noted that a slightly lesser degree of growth was also noted.

Of particular interest with regard to the rate of absorption which would be calculated from the final weight of the pellet is the fact that almost invariably the amount of DCA absorbed from the pellets was greater than calculated either on the assumption that all the loss of weight of the pellets was due to the absorption of active steroid (as suggested by Shimkin 1941),

or calculated by assuming that all the ingredients of the pellet were absorbed in proportion to their initial distribution in the pellet. In every instance in which the material was measured in the 100 per cent pellets, less material was found by the ultraviolet absorption measurement than the weight of the pellet indicated. This difference, of course, is due to the insoluble protein which forms the "ghost" in the pellet. Even in pellets prepared at the same time, containing the same proportions of material and run simultaneously in animals of the same group, there was a distinct difference in the discrepancy which could be ascribed to the formation of the "ghost."

SUMMARY

It was found that when either fused or compressed pellets of DCA and cholesterol were implanted in adrenalectomized male rats, weighing 50 gm. the absorption of the DCA from the pellets and the growth response and survival of the animals were proportional to the percentage of DCA contained in the pellets.

REFERENCES

FUENZALIDA, F.: *Rev. Canad. de biol.* **3**: 366. 1944.

SEGALOFF, A.: *Progress in Clinical Endocrinology* Edited by Soskin, Grune & Stratton, New York 1950, Page 504.

SHIMKIN, M. B., AND J. WHITE: *Endocrinology* **29**: 1020. 1941.

EFFECT OF ADRENAL ENUCLEATION ON SERUM SODIUM*

KATHARINE A. BROWNELL, FRANK A. HARTMAN AND RICHARD W. REIMAN

Department of Physiology, The Ohio State University, Columbus

IS THE glomerular zone of the adrenal cortex the principal source of the sodium hormone? Evidence points that way. After hypophysectomy this zone does not atrophy. Although Nishida (1934), reported a decrease in plasma sodium concentration in the dog after such an operation, Houssay and Mazzocco (1922) found no significant change. Deane and Greep (1946) also observed that steroids and mitochondria persisted in this zone in the rat after hypophysectomy. Desoxycorticosterone caused atrophy of the glomerular zone (Greep and Deane, 1947). Although this synthetic substance is not a hormone of the adrenal cortex, it does cause sodium retention.

If the glomerular zone is responsible for the production of the sodium hormone, enucleation of the adrenals, insofar as it leaves this zone undisturbed, should not greatly modify sodium retention or if it does, the effect should be transient.

In the following experiments we have shown that adrenal enucleation does affect sodium retention, and that this effect lasts long after histological observations (Greep and Deane, 1949) show adequate regeneration.

METHODS

Enucleation of both adrenals in albino rats was carried out by the lumbar path while the animal was under the influence of ethyl ether. All animals were fed Purina chow and given tap water to drink with the exception of one group given 1% sodium chloride. Blood was taken by hypodermic needle from the heart under ether anesthesia. Serum sodium was determined for each animal by the Butler and Tuthill (1931) method. Rats of approximately the same weight that had lived under the same conditions were employed as controls in each group.

RESULTS

Since adrenal insufficiency usually develops within seven days after adrenalectomy we first determined the serum sodium in rats whose adrenals had been enucleated for this period. Nine males weighing 181 to 217 grams showed an average serum sodium level of 152 m.eq./l (range 144 to 156 m.eq./l) while that of three unoperated controls was 145 m.eq./l (range

Received for publication April 17, 1950

* Aided by a grant from the Comly Fund.

144 to 147 m.eq./l). Unfortunately at one time during the period the room temperature reached 38.0°C.

Perhaps seven days is too short a period to reflect much change in serum sodium level. Therefore the remaining groups were kept for a longer time after adrenal enucleation.

Eight groups of rats were maintained for periods ranging from 14 to 29 days. The results are shown in Table 1. Relatively young rats (up to 144 grams weight) two weeks after adrenal enucleation (A and B) showed a serum sodium concentration of 123 m.eq./l for males and 120 m.eq./l for females compared with 137 and 128 m.eq./l respectively for controls. On the other hand older rats (C and D) whose adrenals had been enucleated for two weeks had serum sodium concentrations not significantly different from those of the controls. Three weeks or more after enucleation some rats (E, G and H) showed a significant lowering of serum sodium concentration while others did not (F).

TABLE 1. SERUM SODIUM

	Enucleated rats				Control rats	
Group	Days after enucleation	No. of rats	Weight grams	Serum Sodium m.eq/l	Serum Sodium m.eq/l	No. of rats
A	14	21 ♂	91–144	*123* ± 0.57 (117–132)	*137* ± 0.093 (136–138)	8 ♂
B	14	21 ♀	55–133	*120* ± 0.7 (108–128)	*128* ± 0.28 (121–131)	10 ♀
C	14	17 ♂	210–276	*137* ± 0.41 (130–145)	*131* ± 0.35 (127–135)	7 ♂
D	15	28 ♂	180–295	*133* (127–148)	*135* (129–139)	12 ♂
E	21	25 ♀	89–140	*138* ± 1.24 (108–152)	*145* ± 0.345 (141–151)	8 ♀
F	24	16 ♂	131–184	*127* (107–134)	*125* (107–132)	9 ♂
G	27	24 ♂	134–159	*145* ± 0.69 (134–159)	*152* ± 0.45 (144–157)	8 ♂
H	29	16 ♂	204–280	*135* ± 0.67 (132–140)	*143* ± 0.41 (137–147)	5 ♂

The regenerated adrenals in groups E, F and H were weighed. In the first group (E) the average weight of both adrenals was 9.6 mg. (6.6–17.0 mg.) or 0.0088% (0.0043–0.0162%) of the body weight. According to Donaldson (1924) the adrenals in females at this weight range should be about 0.03% of the body weight (adrenal tissue was found on one side only, in two rats). In the second group (F) the average weight of both adrenals was 6.6 mg. (2.3–9.4 mg.) or 0.0043% (0.0016–0.007%) of the body weight. Donaldson (1924) gives 0.017 to 0.02% for males at this weight (adrenal tissue was found on one side only in eight rats). In the third group (H) the average weight of both adrenals was 17.36 mg. (9.1–29.7 mg.) or 0.0074% (0.0042–0.0162%) of the body weight (adrenal tissue was found on one side only in one rat).

We further reduced the regenerating adrenal tissue by enucleating each adrenal and removing about one half of the capsule. This was done in three groups of male rats. They weighted about 200 gm. when the blood was taken. In the first group (13) animals, a 1% NaCl solution was given for drinking during 23 days and then tap water for 10 days. At the end of this period the average serum sodium was 162 m.eq./l (140–164 m.eq./l)

while normal controls (6) without NaCl had an average serum sodium of 131 m.eq./l (119–141 m.eq./l).

A second group (12) operated in this same way but given no NaCl, at the end of 38 days had an average serum sodium of 140 m.eq./l (131–148 m.eq./l) while the controls (3) had an average of 145 m.eq./l (143–148 m.eq./l). The difference is not significant, considering the small number of controls. A third group (40) similarly operated, at the end of 54 days had an average serum sodium of 135 ± 0.7 m.eq./l (121–148 m.eq./l) while the controls (7) had an average of 140 ± 0.5 m.eq./l (129–143 m.eq./l). This difference is significant since $t = 12$.

EFFECT OF STARVATION

The possibility that deprivation of food and thus sodium chloride for 24 hours might cause a greater decrease in blood sodium in rats with enucleated adrenals than in normal controls led us to try two groups of male animals starved for this length of time.

The first (12 animals), 92 days after enucleation had an average serum sodium of 122 m.eq./l (108–132 m.eq./l) compared with 121 m.eq./l (119-124 m.eq./l) for controls. The second (17 animals), 97 days after enucleation had a serum sodium of 133 m.Eq./l (114–148 m.eq./l) compared with 127 m.eq./l (113–140 m.eq./l) for controls. It will be noted that the sodium values are low but that the sodium concentration in those rats with enucleated adrenals was equal to or above the values for normal animals. In other words adequate adrenal regeneration had occurred. In the second series the average weight of the adrenals was 11.5 mg. (5.9–17.5 mg.) or 0.004% (0.002 to 0.0054%) of the body weight. According to Donaldson (1924) the adrenals should have been about 0.017% of the body weight. (In seven animals the adrenal was missing on one side.)

The condition of all of these rats with enucleated adrenals was excellent with rare exceptions. Only seven died in the whole series, and those during the first week, two in group A, two in group E and three in group F. None of the younger animals lost weight. Instead, they gained remarkably well. Six lost weight in Group D but their serum sodium did not differ from those that gained. One lost weight in Group H, the serum sodium being 132 m.eq./l.

The great range of sodium values should be noted in the enucleated groups having an average sodium level lower than the controls (A, B, E and G).

DISCUSSION

Harrison and Darrow (1938) found in male rats weighing approximately 200 gm. that the serum sodium averaged 136 m.eq./l (131–140 m.eq./l)

when insufficiency symptoms had developed after adrenalectomy (within 5 days). Control animals had an average of 144 m.eq./l (140–146 m.eq./l) of serum sodium.

We found it necessary to run controls at the same time as the experimental animals because of variations in temperature and other conditions. Age also appears to be important.

Our results show that adrenal enucleation in young rats is often followed by a reduction in serum sodium concentration whereas older rats may show little change. The young animals would seem to be the best test objects for adrenal function since it is well known that adrenal insufficiency is more easily produced in them.

It should be noted that rats maintain their serum sodium level very poorly. Twenty-four hours' starvation caused marked reduction in these levels in normal rats as well as in those with adrenals enucleated three months before. In other words variation in food intake for only a day or so would produce considerable variation in serum sodium. Also with excess sodium intake as in the animals given 1% NaCl to drink the serum sodium remained at a high level for longer than ten days after changing to tap water.

It is impossible to determine to what extent the glomerulosa was reduced by enucleation. In most instances enough active cortical tissue remained to maintain growth. However, in isolated cases we assume that the blood supply was sufficiently disturbed to cause complete degeneration on one side since no gland was found there. It has also been suggested that the regenerating glomerulosal cells do not at first secrete and that this might account for reduced secretory activity for a time following enucleation. This insufficiency persisted in some individuals after a considerable amount of regeneration had occurred and could be explained by insufficient activity of inner zones. Our experiments suggest the latter explanation but do not prove it. Greep and Deane (1949) observed that a new cortex develops rapidly in the rat, an "essentially normal cortex" being completed by the end of a month. Despite this in our animals serum sodium level has not always returned to normal 54 days after enucleation.

Our experiments demonstrate that in young adult albino rats, at least, when much of the inner zones of the adrenal cortex is destroyed, plasma sodium tends to fall. Whether this is due to glomerulosa zone insufficiency alone or also involves inner zones has not been established.

SUMMARY

The effect of adrenal enucleation on the serum sodium concentration of young and old male and female rats was studied.

Serum sodium in rats depends upon sodium intake and is increased by

high temperature. Therefore controls must always be run simultaneously with experimental animals under the same conditions.

Serum sodium concentration falls and remains below normal for a few weeks following adrenal enucleation which removes a large proportion of the cortices, indicating that the tissue removed is necessary for maintenance of normal serum sodium concentration, that remaining being inadequate. That this is not due to glomerulosa zone insufficiency alone but involves other zones is suggested but not established by our data.

REFERENCES

BUTLER, A. M., AND E. J. TUTHILL: *J. Biol. Chem.* **93**: 171, 1931.
DEANE, H. W., AND R. O. GREEP: *Am. J. Anat.* **79**: 117, 1946.
DONALDSON, H. H.: The Rat, 2nd Ed. Memoirs Wistar Inst. Phila., 1924.
GREEP, R. O., AND H. W. DEANE: (1) *Endocrinology* **40**: 417, 1947.
GREEP, R. O., AND H. W. DEANE: (2) *Endocrinology* **45**: 42, 1949.
HARRISON, H. E., AND D. C. DARROW: *J. Clin. Investigation* **17**: 77, 1938.
HOUSSAY, B. A., AND P. MAZZOCCO: *Comp. rend. soc. biol.* **86**: 409, 1922.
NISHIDA, S. SEI-I-KAI: *Med. J.* **53**: 133, 1934.

β-GLUCURONIDASE ACTIVITY IN THE RAT UTERUS[1]

SAMUEL L. LEONARD AND ERNEST KNOBIL[2]

From the Department of Zoology, Cornell University, Ithaca, New York

MANY animal tissues contain an enzyme β-glucuronidase, which is specific for the hydrolysis of β-glucuronides *in vitro* (Oshima, 1934). It has been observed (Fishman and Fishman, 1944; Fishman, 1947a; Kerr, Campbell and Levvy, 1949) that the activity of β-glucuronidase in uterine tissue of ovariectomized mice is significantly less than that of normal animals, and that this activity could be returned to the normal level by the administration of estrogens in physiological doses. These findings led to the supposition that a relationship exists between the activity of uterine β-glucuronidase and the level of estrogenic hormones in the animals studied. Although the exact nature of this relationship remains to be determined, it was thought that a study of uterine β-glucuronidase activity might provide qualitative and quantitative information concerning normal variations in estrogen levels as they occurred in the rat.

In the present paper, results of experiments dealing with changes in uterine β-glucuronidase in the estrous cycle, after ovariectomy and estrogen administration, are reported.

METHODS

β-glucuronidase was assayed by the method of Talalay, Fishman and Huggins (1946). This method involves the colorimetric determination of phenolphthalein liberated by the hydrolytic action of the enzyme on a biosynthetically prepared substrate, phenolphthalein mono-β-glucuronide (Fishman, Springer and Brunetti, 1948). The unit of β-glucuronidase activity is defined as that amount of enzyme which will liberate one gamma of phenolphthalein in one hour at 38°C. from phenolphthalein glucuronide at pH 4.5 under standard conditions (Talalay *et al.*, 1946). The results in the following experiments will be expressed as enzyme units per mg. of wet weight and mg. of dry weight of tissue. The enzyme activity per mg. wet weight of tissue is reported for purposes of comparison with previously published reports. It would seem that enzyme activity reported as units of β-glucuronidase per mg. of tissue, dry weight, more closely approximates actual changes, since fluctuations in uterine water content, known to be affected by the administration of estrogens, can thus be eliminated as a source of error in the interpretation of the data. The conclusions reported in this communication are therefore based on β-glucuronidase activity expressed as units per mg. of uterine tissue, dry weight.

Received for publication April 24, 1950.

[1] Aided by a grant from the Sage Research Fund.

[2] Fellow of the Schering Corporation.

The animals in any one experimental group were selected for comparable age and weight. Female rats were first anesthetized with ether then killed with a sharp blow on the head and allowed to bleed. The uteri were removed, trimmed of adhering fat and connective tissue, and placed in a chilled, 0.9% saline solution. All steps in the procedure prior to incubation were carried out in the cold where possible. The uteri were slit longitudinally with fine scissors to expel the uterine fluid when present, blotted dry on paper toweling and quickly weighed on a torsion balance. A small portion of the uterus was dried to constant weight at 90–100°C. for the determination of water content. The sample of uterine tissue to be assayed for β-glucuronidase activity was homogenized in a glass Potter homogenizer, the concentration of the homogenates being adjusted to approximately 1% with distilled water. The homogenates were centrifuged for fifteen minutes at 1300 RPM and 0.5 ml of the resulting supernatants were incubated with the substrate at a pH of 4.5 for one hour at 38°C. At the end of this period the reaction was stopped and the color developed by the addition of glycine buffer at pH 10.45. The solutions were centrifuged for 10 minutes to remove precipitated proteins. The clear, pink supernatants were read in a Klett-Summerson Photoelectric Colorimeter using a 540 mμ filter, and the readings compared with a standard phenolphthalein curve.

EXPERIMENTAL

Changes of β-glucuronidase in the estrous cycle. In view of the fact that Fishman (1947a) obtained an increase in β-glucuronidase activity in the uteri of ovariectomized mice on administration of as little as 0.025 gamma of estradiol, it was thought that changes in enzyme activity might be expected in the course of the estrous cycle in the rat.

TABLE 1. UTERINE β-GLUCURONIDASE ACTIVITY IN THE ESTROUS CYCLE

Stage of cycle	Number of rats	Uterine weight mg.	Units of β-glucuronidase	
			Per mg. wet wt.	Per mg. dry wt.
Females in estrus	16	410.5 ±34.6[1]	7.42 ±0.43[1]	48.48 ±8.40
60 hours post estrus	14	344.8 ±23.3	7.30 ±0.47	41.31 ±3.77

[1] Results in this and subsequent tables expressed as means and their standard errors.

Female rats were selected for estrus by the vaginal smear technique followed by testing for lordosis with males, between 7 and 10 P.M. One half of the animals thus selected were assayed for uterine β-glucuronidase that same evening or early the next morning. The remaining animals were assayed approximately sixty hours later. The uteri of the rats in heat were in every case distended with fluid while those of the rats examined 60 hours later were flaccid and in the typical diestrous condition. These two stages in the cycle were assumed to represent the extremes in the estrogen levels.

The β-glucuronidase levels of the uteri of these rats showed no significant differences at these stages of the cycle (Table 1). This was true when the

results were calculated on the basis of wet weight or dry weight of tissue. It should be emphasized at this point that large variations in β-glucuronidase activity were encountered among the animals of any given experiment in spite of the fact that all procedures were carried out as carefully as possible. The water content of the uteri at the stages of the cycle studied exhibited no significant difference. It was noted that the difference of uterine weights between the animals in estrus and those in diestrus was not a significant one ($P = >.1$) when the uterine fluid was expelled prior to weighing of the organ.

Reduction of β-glucuronidase activity with ovariectomy. Failure to detect a difference between the uterine β-glucuronidase activity of female rats in estrus and diestrus might be explained in two ways: 1) that the enzyme activity changed significantly but that these changes were transitory and hence not detectable in the experiment, or 2) that there was no change in β-glucuronidase activity in the period comprised by the experiment, i.e., 60 hours. The following experiment, designed to determine the time necessary to effect a significant loss of enzyme activity after ovariectomy, validates the second hypothesis.

Female rats were ovariectomized and their uteri assayed for β-glucuronidase activity 2, 4, 6, 8, 10, 12, 20, 30, and 270 days after the operation. The results are shown in table 2. In comparing the enzyme levels of the castrated rats with those of their normal controls, it was found that a

TABLE 2. LOSS OF UTERINE β-GLUCURONIDASE AFTER OVARIECTOMY

Days after castration	Number of rats	Body weight gm.	Uterine weight mg.	Units β-glucuronidase	
				Per mg. wet wt.	Per mg. dry wt.
0	7	214.3	250.5 ± 40.0	8.16 ± 0.98	37.96 ± 4.53
2–6	11	223.4	157.2 ± 11.8	8.23 ± 0.61	38.60 ± 3.01
8–12	9	214.4	74.6 ± 7.3	5.30 ± 0.52	24.42 ± 1.57
20	9	222.4	68.1 ± 4.2	2.80 ± 0.28	13.40 ± 1.20
30	4	229.8	47.2 ± 6.6	1.80 ± 0.39	—
270	6	341.8	47.3 ± 3.2	1.90 ± 0.05	10.80 ± 2.80

significant loss of enzyme activity occurred only 8–12 days following ovariectomy ($P = <.05$). At this time, however, the decrease in uterine weight was highly significant ($P = \ll.01$). The decrease in enzyme activity between 8–12 days and 20 days following ovariectomy was greater than that observed between 0 and 8–12 ($P = \ll.01$), although no significant loss of uterine weight was noted during this period ($P = >.4$). The lowest level of β-glucuronidase was attained 20–30 days after ovariectomy, no significant difference being observed between 20 and 270 days after the operation.

β-glucuronidase after estrogen treatment. Rats ovariectomized for 20–23 days were injected subcutaneously with 0.83, 4.15, 20.75 and 41.5 gamma of estradiol benzoate[3] in oil. These animals were assayed for β-glucuronidase approximately 72 hours after a single injection. The enzyme response to the administration of estradiol benzoate is summarized in table 3.

The smallest dose administered, 0.83 gamma, gave a significant increase in enzyme activity over the 20-day ovariectomized controls ($P = \ll .01$). The increase effected by the larger doses above that of the smallest dose was very slight, and not significant. In no instance did the actual amount equal that present in a rat with intact ovaries.

TABLE 3. OVARIECTOMIZED RATS ASSAYED 72 HRS. AFTER SINGLE INJECTION OF ESTRADIOL BENZOATE

Dose of estrogen gamma	Number of rats	Body weight gm.	Uterine weight gm.	Units β-glucuronidase	
				Per mg. wet wt.	Per mg. dry wt.
0[1]	9	222.4	68.1 ± 4.2	2.80 ± 0.28	13.40 ± 1.20
0.83	4	248.5	166.6 ± 37.8	3.77 ± 0.32	17.32 ± 1.39
4.15	4	254.8	207.4 ± 35.0	4.13 ± 0.57	21.62 ± 2.22
20.75	4	227.0	202.1 ± 3.7	4.63 ± 0.46	26.97 ± 2.09
41.50	4	224.5	210.5 ± 23.0	4.27 ± 0.42	22.65 ± 1.56

[1] Data from 20 day castrates, Table 2.

In the next experiments, rats ovariectomized for 20 days were assayed for β-glucuronidase activity 12, 24, 48, and 72 hours after a single injection of 41.5 gamma of estradiol benzoate in oil. As can be seen from the data in table 4, a single injection of the hormone effected an initial drop in the concentration of uterine β-glucuronidase followed by a gradual rise in enzyme activity which became statistically significant 48 hours after the injection ($P = < .02$). Despite the fact that the unexpected decrease in β-glucuronidase activity noted 12 hours after estrogen administration was not statistically significant, ($P = > .05$) when calculated on the basis of dry weight, it was relatively consistent in most of the experimental animals, (10 out of 12) and may represent an actual physiological change. It was also noted that a significant increase in uterine weight occurred at the time when the decrease in enzyme activity was observed.

Finally, an experiment was performed to see if it were possible to raise the β-glucuronidase level above that found in a normal female rat. Ten female rats were ovariectomized and injected immediately with 8.3 gamma of estradiol benzoate and the treatment continued daily for 20 days. The ovariectomies were performed in order to avoid possible interference from

[3] The estradiol benzoate (Progynon B) was kindly supplied by the Schering Corporation.

TABLE 4. OVARIECTOMIZED RATS ASSAYED AT INTERVALS AFTER A SINGLE INJECTION OF 41.5 GAMMA ESTRADIOL BENZOATE

Hours after injection	Number of rats	Body weight gm.	Uterine weight mg.	Units β-glucuronidase	
				Per mg. wet wt.	Per mg. dry wt.
0	17	222.8	76.9 ± 3.7	2.78 ± 0.17	13.12 ± 0.81
12	12	225.1	128.0 ± 8.4	1.80 ± 0.13	10.02 ± 1.13
24	12	226.7	138.1 ± 8.1	2.42 ± 0.22	14.66 ± 1.17
48	8	241.9	177.3 ± 14.5	2.72 ± 0.12	16.26 ± 0.80
72	8	224.8	223.1 ± 13.1	3.97 ± 0.16	22.52 ± 0.96

progesterone, produced by persistent corpora lutea which would form if intact animals were subjected to prolonged estrogen treatments. β-glucuronidase activity, when expressed on the basis of dry weight, was significantly higher ($P = .01$) than in the normal unoperated animals. The results are shown in table 5.

DISCUSSION

The foregoing experiments clearly indicate that uterine β-glucuronidase activity could not be used as an index of physiological changes in estrogen levels during the normal estrous cycle in the rat. The concentration of this enzyme in uterine tissue appeared to remain relatively constant despite

TABLE 5. 20 DAY TREATMENT WITH DAILY DOSE OF 8.3 GAMMA ESTRADIOL BENZOATE, STARTED DAY AFTER OVARIECTOMY

Experiment	Number of rats	Body weight gm.	Uterine weight mg.	Units β-glucuronidase	
				Per mg. wet wt.	Per mg. dry wt.
Controls[1]	7	214.3	250.5 ± 40.0	8.2 ± 0.98	37.96 ± 4.53
Estrogen treated	10	174.9	246.0 ± 14.7	9.9 ± 0.50	52.70 ± 2.30

[1] Data from 0 day castrates, table 2.

the physiological and anatomical changes of the uterus during the cycle. In order to demonstrate changes in the enzyme level it required that either the animal must be deprived of its ovaries for an extended period of time or that adequate dosages of estrogen must act in the castrated animal for at least a period between 48 and 72 hours. It is quite probable that the normally short estrous cycle of the rat did not allow sufficient time for alterations in the enzyme level since, in a few preliminary experiments, the enzyme concentration of the uterus of rats made pseudopregnant was definitely lower (by the 10th day) than that obtained at the regular stages of the cycle. Further work along this line is in progress.

When estrogens were administered to the ovariectomized rats the uterine

β-glucuronidase activity could not be correlated, on a parallel time basis, with uterine growth. The increase in uterine weight 12 hours after hormone injection was highly significant ($P = \ll .01$) whereas no parallel increase in the enzyme level was seen, and indeed, a slight drop occurred (see below). This increase in uterine weight preceded a significant increase in β-glucuronidase concentration by 36 hours. Furthermore, the mean uterine weight (223 mg.) of the injected animals 72 hours after treatment approximated that of intact animals (250 mg.) of similar age and body weight although the enzyme concentration at this time had returned to only slightly over half the normal level.

A similar relationship was noted in the converse experiment where it was observed that the decrease in uterine weight of ovariectomized animals did not parallel the decline in β-glucuronidase activity. For example, a significant decrease in enzyme level occurred between 8–12 and 20 days following ovariectomy without a concomitant loss of uterine weight.

The decrease in uterine β-glucuronidase which occurred 12 hours after the injection of 41.5 gamma of estradiol benzoate into females ovariectomized for 20 days cannot be satisfactorily explained at this time. Although results based on enzyme units per mg. wet weight have not been emphasized in this report, it should be mentioned that β-glucuronidase activity, when expressed in this manner (table 4), showed a statistically significant decrease 12 hours after the single estrogen injection, ($P = \ll .01$) but was not statistically significant on the dry weight basis. Furthermore, a rise in enzyme activity, when expressed on the basis of wet weight is not evident before 72 hours. It is highly probable that these discrepancies between the dry and wet weight values may be explained in terms of an altered water content in the uteri of the estrogen-treated rats.

Although it was possible to effect a significant increase in the enzyme level above that observed in intact females after prolonged estrogen treatment, the magnitude of this increase was not great. One might conclude from this that uterine β-glucuronidase in the adult rat remains near a maximum under normal conditions during the estrous cycle.

At present there is no satisfactory explanation for the function of β-glucuronidase in the uterus of mammals. Whatever the explanation may be, the lag between alterations in uterine morphology and the appearance of altered enzyme levels must be taken into consideration. Fishman (1947a) proposed that β-glucuronidase catalyzes the conjugation of estrogens with glucuronic acid and that this process was not merely a detoxication mechanism, but one of "metabolic conjugation" which plays a fundamental role in the utilization of the estrogenic hormone by the tissue. Karunairatna, Kerr and Levvy (1949) however, present ample evidence to show that β-glucuronidase plays no part in glucuronide synthesis and that the en-

zyme system mediating this reaction is separate and distinct from β-glucuronidase. Levvy, Kerr and Campbell (1948) and Kerr, Campbell and Levvy (1949) concluded that a rise in β-glucuronidase is associated with active cell proliferation and is therefore an index of general tissue growth. This concept is supported by observations (Fishman, 1947b; Fishman and Anlyan, 1947) that cancer tissue possesses a higher activity of β-glucuronidase than normal control tissues.

SUMMARY

During the estrous cycle of the rat no change in the uterine β-glucuronidase activity was found. A decrease in β-glucuronidase activity was not observed before 8–12 days following ovariectomy; a further decrease in the enzyme level occurred without a concomitant decrease in uterine weight. A single injection of estradiol benzoate into ovariectomized rats resulted in (1) a consistent although not statistically significant decrease in enzyme activity and (2) an increase in uterine weight 12 hours after estrogen administration. This was followed by a gradual rise in enzyme concentration which reached a significantly higher level 48 hours after the injection. Prolonged estrogen treatment of castrated rats increased the enzyme level slightly, but significantly, above that of normal intact rats.

REFERENCES

FISHMAN, W. H., AND L. W. FISHMAN: *J. Biol. Chem.* **152:** 487. 1944.
FISHMAN, W. H.: *J. Biol. Chem.* **169:** 7. 1947a.
FISHMAN, W. H.: *Science* **105:** 646. 1947b.
FISHMAN, W. H. AND A. J. ANLYAN: *Cancer Res.*: **7:** 808. 1947.
FISHMAN, W. H., B. SPRINGER AND R. BRUNETTI: *J. Biol. Chem.* **173:** 449. 1948.
KARUNAIRATNAM, M. C. AND G. A. LEVVY: *Biochem. J.* **44:** 599. 1949.
KARUNAIRATNAM, M. C., L. M. H. KERR, AND G. A. LEVVY: *Biochem. J.* **45:** 496. 1949.
KERR, L. M. H., J. G. CAMPBELL AND G. A. LEVVY: *Biochem. J.* **44:** 487. 1949.
LEVVY, G. A., L. M. H. KERR, AND J. G. CAMPBELL: *Biochem. J.* **42:** 462. 1948.
OSHIMA, G.: *J. Biochem., Japan*, **20:** 361. 1934.
TALALAY, P., W. H. FISHMAN AND C. HUGGINS: *J. Biol. Chem.* **166:** 757. 1946.

ADRENAL CORTICAL HORMONE LEVELS IN ADRENAL VEIN—AND PERIPHERAL BLOOD*

K. E. PASCHKIS, A. CANTAROW, A. A. WALKLING AND D. BOYLE

Division of Endocrine and Cancer Research, Jefferson Medical College, Philadelphia, Pennsylvania

THERE is but little information available regarding the rate of secretion of adrenal cortical hormones, their levels in the blood and their ultimate metabolic fate. This communication deals with experiments undertaken to determine the amount of hormone in the blood of the adrenal vein and in the peripheral circulation. Inasmuch as the assay method employed was that based upon the effect of glycogen synthesis, the values obtained may be considered to indicate the levels of C-11-oxygenated-21 carbon steroids (11-oxysteroids).

MATERIALS AND METHODS

All blood samples, except as indicated, were obtained from trained dogs, eliminating, insofar as possible, the factors of fear and excitement as "alarming stimuli." Peripheral arterial blood was drawn from the femoral artery, peripheral venous blood from the antecubital vein. In order to obtain blood from the adrenal vein under comparable conditions, a modified London cannula was employed. The cannula, made of vitallium, was of the type employed by Crandall (Crandall, *et al.* 1947); two eyes at the end of the cannula permitted it to be sutured in the muscle, fixing it above the lumboadrenal vein.

Plasma was separated from blood drawn into syringes containing small amounts of heparin.

The mouse liver glycogen assay method of Venning (Venning 1946) was employed. Plasma was diluted with glucose and alcohol in the same way Venning dilutes the urinary extracts with glucose and alcohol. Each mouse received 7 injections of 0.2 ml. of plasma, a total of 1.4 ml. A dose response curve obtained with 11-dehydrocorticosterone acetate (Compound A acetate) was used as standard. Results are expressed as microgram equivalents of compound A acetate.

4 to 8 mice were used for each assay. Because of the slope of the log, dose-response curve, even small differences of the microgram equivalent values of compound A represent much greater differences of liver glycogen. In order to conserve space the raw data are not given for every experiment, but the following will serve as example. In experiment 2, table 3 the rise of cortical hormone in plasma of the dog following injection of epinephrine was from 12.1 to 15.3 microgram equivalent of compound A, a rise of 3.2

Received for publication May 19, 1950.

* This work was supported by a grant-in-aid from the Ciba Corporation.

Part of this work has been reported at the meetings of the Federation of Biological Societies 1948 and 1949 (Paschkis, *et al.* 1948, 1949).

micrograms (26%). The corresponding liver glycogen values in the assay mice were 7.9 and 15.9 mgm per 100 gm bodyweight; this difference is significant ($p<0.05$). In experiment 2, table 2 the cortical hormone in the plasma of the dog rose, following injection of ACTH, from 11.9 to 12.8 microgram equivalent of compound A, a rise of 0.9 microgram (7%). The corresponding mouse-liver glycogen values are 7.2 and 11.1 mgm. per 100 gm. body weight; the difference is significant ($p<0.05$).

Control Experiments

It was necessary to ascertain whether the small amounts of glucose contained in the plasma injected into the adrenalectomized assay mice would appreciably influence the liver glycogen. It was found that the latter was not significantly increased above the control level following injection of 1.4 ml. of a 0.1 per cent glucose solution.

In certain experiments in which hormone assays in peripheral blood were used for assay, up to 40 ml. of blood were withdrawn several times during an experiment. In order to ascertain whether repeated bleeding of this magnitude might influence cortical hormone levels, control experiments were performed which demonstrated that withdrawal of 40 ml. of blood did not cause an increase in plasma hormone levels.

RESULTS AND DISCUSSION

Cortical Hormone in Adrenal Vein Blood

M. Vogt (Vogt 1943; 1947) studied the hormone content of adrenal vein blood, using the Schenker-Selye cold test as an assay procedure. She used anesthetized dogs with the adrenals exposed. The levels obtained were high; the standard of reference was a commercial adrenal extract, and the values were expressed as equivalents of grams of adrenal tissue from which the commercial extract was prepared. Under these experimental conditions, the dogs were obviously studied in a state of severe stress incident to the operative procedure and anesthesia.

Our dogs were used several weeks after operation, were trained and blood was obtained through the London cannula under as nearly physiological conditions as possible. The values obtained in four dogs were 14, 20, 20, and 50 microgram equivalents of Compound A acetate respectively per ml. of plasma. The highest value, 50 micrograms, was obtained in a dog in whom difficulty was encountered in obtaining blood through the cannula and who showed considerable excitement and struggled during the procedure.

An exact comparison of these values with those obtained by Vogt is obviously impossible. A different assay method was employed. The mouse-liver glycogen assay is specific for C-11 oxygenated compounds whereas the results of the cold test employed by Vogt may be influenced by 11-desoxycorticosterone-like compounds also (Dorfman 1946). Moreover, our standard of reference was crystalline 11-dehydrocorticosterone acetate, and the results are expressed in microgram equivalents of the latter, whereas Vogt's standard of reference was a commercial aqueous cortical

extract (Eucortone), her results being expressed in equivalents of grams of adrenal tissue extracted.

In order to obtain an admittedly roughly approximate comparison, we have recalculated our results in the following manner: 1 ml. of a commercial adrenal extract (Upjohn aqueous extract) was assayed against Compound A acetate and was found to have an activity of about 360 microgram equivalents of Compound A acetate per ml. According to a personal communication from Dr. Kuizenga, 1 ml. of the Upjohn aqueous adrenal cortical extract represents 40 grams of adrenal cortical tissue. The values obtained by us ranged therefore from 1.5 to 6.9 gram equivalents of adrenal cortical tissue per ml. of adrenal vein plasma. These values, although lower than those of Vogt's, are of a similar order of magnitude. Vogt calculated 1 ml. of plasma to correspond to 12.5 gm. of cortical tissue. The difference may be due in part to the fact that cortical extracts of different manufacture were used; furthermore, different assay methods were employed, and above all, the condition of the animals differed widely. We believe that the absence of alarming or stress factors under the conditions of our experiments is of great importance in this connection.

Hormone Levels in Peripheral Blood

Hormone could be demonstrated consistently in the peripheral arterial blood of untreated trained dogs (Table 1). In Venning's assay procedure,

TABLE 1. LIVER GLYCOGEN AND MICROGRAM EQUIV. OF CP. A AC. IN ARTERIAL PLASMA OF UNTREATED DOGS

	Number of mice	Liver Glyc. mg./100 g. body wt.	Equiv. Cp. A Ac. in 1 ml.
Adrenalect. Control Mice	59	6.2 ± 1.76 mg.*	"11.6"
Injected 1.4 ml. dog plasma	60	7.4 ± 1.05 mg.*	12.1
Difference		1.2	
p of Diff.		<0.001	

* Standard Deviation.

the control mouse has a small, rather constant liver glycogen content, resulting from the glucose administered as part of the procedure. The increase in liver glycogen of mice treated with 1.4 ml. of dog arterial plasma over that of the control mice is small, but the difference is highly significant. In Table 1 the results of a number of experiments, done at different times, were pooled. In individual experiments, using 4 to 8 mice, the variations in liver glycogen from one animal to another were small enough to make the values significant. Three experiments are presented as typical

examples. The mean liver glycogen content of the liver (mg per 100 gm body weight) of 4 mice in each experiment was 7.66 ± 0.39,[1] $7.09^1 \pm 1.06$,[1] 8.30 ± 1.01^1 respectively.

The presence of adrenal cortical hormone in blood has been demonstrated heretofore only in cases of Cushing's Syndrome resulting from hyperfunction of the adrenal cortex. The assay method employed was based on growth and survival of adrenalectomized rats (Anderson and Haymaker 1938). Vogt, using the Schenker-Selye cold test, was unable to detect adrenocortical hormone in the peripheral blood of normal animals.

Cortical Hormone in Blood Following Injection of ACTH and of Epinephrine

Following intravenous injection of ACTH (Armour)[2] the hormone level in the peripheral arterial blood rises (Table 2). It would appear that within

TABLE 2. ADRENAL CORTICAL HORMONE IN ARTERIAL PLASMA OF DOGS FOLLOWING I.V. INJECTION OF ACTH

Exp. No.	ACTH mg.	Microgram equiv. of Cp. A Ac. per ml. plasma		Hours after injection
		Before	After ACTH	
1	15	12.1	15.3	4
2	10	11.9	12.8	4
3	20	12.3	14.2	5
4	10	11.7	14.0	6
5	10	12.8	15.0	4

the range of dosage of ACTH employed, the magnitude of the response does not depend on the dose.

There is ample evidence in the literature (Long 1947, Thorn and Forsham 1949) that injection of epinephrine causes the anterior pituitary to release ACTH. This mechanism has been demonstrated indirectly by study of the peripheral effects of the cortical hormone, as well as by chemical changes in the adrenal glands (ascorbic acid, cholesterol). Data presented in Table 3 indicate the increase in circulating cortical hormone that follows injection of epinephrine.

Influence of Alarming Stimuli Upon Circulating Cortical Hormone

It is now recognized that the adrenal cortex responds with increased secretion of hormone to a great variety of stresses or alarming stimuli. Evidence has come from study of peripheral effects of the hormones, from chemical changes in the adrenal gland, and from studies of urinary excretion of corticoids in the urine of humans. Table 4 contains data on the

[1] σ = Standard deviation.

[2] ACTH was supplied by Armour and Co., through the courtesy of Dr. J. R. Mote.

TABLE 3. ADRENAL CORTICAL HORMONE IN ARTERIAL PLASMA OF DOGS FOLLOWING INJECTION OF EPINEPHRINE

Exp. No.	Epinephrine mg./kg.	Microgram equiv. of Cp. A Ac. per ml. plasma		Hours after injection
		Before	After	
1	0.02 i.v.	12.1	13.5	1
2	1 mg. sc. 4 times*	12.1	15.3	1**
3	1 mg. s.c. 3 times*	12.8	15.0	1**
4	2 mg. s.c. 4 times*	12.8	14.0	1**

* Injection at one hour interval.
** 1 hour after last injection.

effect of the injection of formalin, colchicine and insulin. All of these stimuli led to a rapid increase in circulating cortical hormone, but of different degree. Colchicine has been reported to be a particularly powerful alarming stimulus in rats (Leblond *et al.*, 1938, Seifter *et al.*, 1949). In the dogs, the levels of cortical hormone did not increase as much following colchicine as following the other stimuli studied. The dose employed was sufficiently large to cause death of the dogs during the night following the experiment, but the animals were in apparently good condition when the blood samples were obtained.

TABLE 4. CORTICAL HORMONE LEVELS IN ARTERIAL BLOOD OF DOGS FOLLOWING STRESS

Exp. No.	Agent dose	Microgram equiv. of Cp. A Ac. per ml. plasma		Hours after injection
		Before	After	
1	I 3	12.1	18.5	2¾
2	I 3	12.2	21.4	2¾
3	I 3	12.3	15.0 (12.8)	3¾ (24)
4	I 0.6	12.1	14.6	1½
5	I 0.23	12.5	13.5	1
6	I 0.5	11.9	12.8	2¾
7	Fo 20 ml.* 4 times	12.1	15.7	48
8	Fo 40 ml.	11.5	16.7	3½
9	Co 1	11.8	12.8	4
10	Co 1	—	13.5 (12.8)	4 (8)

I : Insulin intraven., u/kg.
Fo: 10% Formalin intramusc.
Co: Colchicine subcut., mg./kg.
* 20 ml. Fo injected twice daily on day one and two, blood on day 3, 48 hours after first, about 17 hours after last injection.

Two alternative explanations for this small rise suggest themselves. Colchicine may have a dual action, one involving stimulation of cortical hormone secretion (alarm reaction), the other, perhaps associated with its general carioclastic activity, not involving the adrenal cortex. Alterna-

tively, it might be assumed, that a very powerful "poison" might cause a large demand for, and a highly increased utilization of, adrenocortical hormone, so high that increased secretion by the gland could not keep pace with utilization. This explanation would assume that adrenocortical hormone is in some way utilized in conditions of stress. It must, however, be pointed out that this has never been demonstrated. Experiments bearing on this point are now under way in our laboratories.

The highest blood levels, under the experimental conditions employed, were observed after injection of insulin. In no instance was there any clinical evidence of hypoglycemia. The lowest blood sugar level recorded was 41 mg. per 100 ml., but since blood sugars were determined only before injection of insulin and again at the end of the experiment, one to three and three quarter hours after injection, it is possible that the point of maximum depression of blood sugar was missed. No attempt can be made, therefore, to correlate the degree of hypoglycemia with the degree of rise of cortical hormone level.

TABLE 5. CORTICAL HORMONE LEVELS IN ARTERIAL PLASMA AFTER I.V. INJECTION OF DIBENAMINE (10 MG./KG.)

	No. of exp.	Microgram equiv. of Cp. A Ac. per ml. plasma
Normal Plasma	10	12.1 ± 0.73*
After Dibenamine**	8	14.2 ± 0.58*

*Standard deviation.
** Blood was drawn 1 to 48 hours after Dibenamic Injection.

Shrinkage of lymphatic tissue, indicating stimulation of the adrenal cortex, has been observed following insulin injection in rats (Zeckwer 1948).

The fact that insulin-induced hypoglycemia appears to be a powerful stimulus for secretion of adrenal cortical hormone may conceivably have some bearing on the interpretation of results of insulin shock therapy.

Experiments with Dibenamine

In view of Long's theory that various alarming stimuli act by inducing release of epinephrine, which in turn "fires" the anterior pituitary to release ACTH, it was decided to investigate the effect of sympathetic blocking ("sympathicolytic") agents upon the blood level of cortical hormone following an alarming stimulus. N,n-dibenzyl-β-chlorethylamine (Dibenamine) was employed for this purpose in the majority of experiments. Data presented in Table 5 indicate that i.v. injection of Dibenamine (10 mg./kg. body weight) in every case caused an increase in circulating cortical hormone. It, therefore, of itself constitutes an alarming stimulus. The rise in

the level of cortical hormone was observed as early as one hour after injection, and persisted for as long as 48 hours. The finding that Dibenamine itself causes an alarm reaction is in agreement with the indirect evidence reported by Tepperman *et al.* (1948), and by Seiffter *et al.* (1949). A few experiments with other adrenolytic drugs (unpublished) suggest that this alarming action is not limited to Dibenamine, but a more extensive survey is necessary and is at present under way.

The data presented in Table 6 indicate that an alarming stimulus in a dog pretreated with Dibenamine induces a smaller rise in cortical hormone levels than in animals not so pretreated. This becomes evident on comparing these data with those in Table 4. Results are summarized in Figure 1.

TABLE 6. INFLUENCE OF DIBENAMINE ON RESPONSE OF CORTICAL HORMONE LEVELS TO INSULIN AND FORMALIN

Exp. No.	Dibenamine mg./kg. i.v	Plasma		Hours after Diben.	Insulin i.v. u/kg.	Plasma		Hours after Insulin
		Glucose*	Hormone**			Glucose*	Hormone**	
1	10	102	13.7	24	3	57	15.7	2¾
2	10	97	14.3	22	3	42	17.1	2½
3	10	101	13.2	48	3	66	14.6	2¾
4	10	100	14.2	22	3	55	16.3	2¾
5	10	97	14.1	19	0.4	70	14.6	2
					Formalin 10% S.C.			
6	10	99	15.0	25	40 ml.	110	13.7	2

* Mg./100 ml.
** Cortical Hormone, Microgram Equiv. of Cp. A Ac. per ml.

Thus in animals pretreated with Dibenamine, subsequent injection of insulin (i.v.) or formalin (s.c.) was followed by a smaller rise in cortical hormone levels than was observed in dogs not pretreated with the sympathetic blocking agent. Following insulin (3 u per Kg.) the average rise was 50%, whereas in dogs receiving the same amount of insulin but pretreated with Dibenamine, the rise was only 15% above the level induced by Dibenamine and present at the time of injection of insulin. Formalin produced no increase in cortical hormone levels over that induced by Dibenamine.

Epinephrine causes increased secretion of adrenal cortical hormone, as has been demonstrated by studies of ascorbic acid and cholesterol content of the adrenal (Long *et al.*, 1945) as well as of circulating lymphocytes and eosinophiles (Thorn *et al.*, 1949). An increase in the level of circulating cortical hormone following injection of epinephrine is indicated in data presented in Table 4. C. N. H. Long has proposed the theory that other alarming stimuli act by first causing release of epinephrine from the adrenal medulla, which in turn "fires" the anterior pituitary to release ACTH; the

latter then induces secretion of hormones from the adrenal cortex (Long, 1947).

Sayers and Sayers, on the other hand, believe that secretion of ACTH from the anterior pituitary is determined by the demand of the body for adrenal cortical hormone, or by the levels of cortical hormone in the blood and body fluids (Sayers, 1948, 1949).

Our findings would be compatible with either theory. The smaller rise

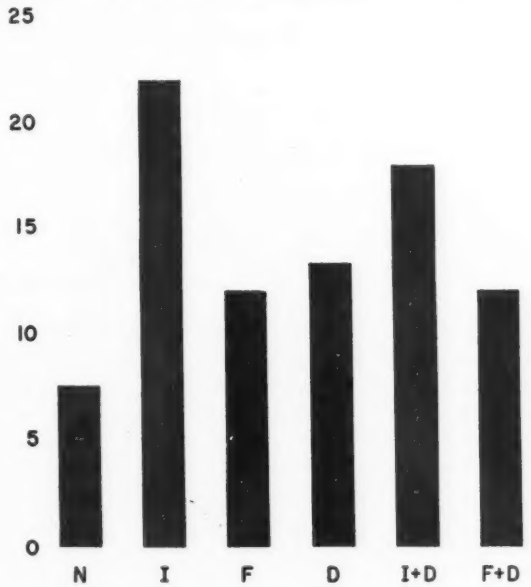


FIG. 1. Mean mouse liver glycogen (mg. per 100 mg. body weight). Mice injected with dog plasma: N—Normal Controls; I—Insulin treated; F—Formalin treated; D—Dibenamine treated; I+D—Insulin after Dibenamine; F+D—Formalin after Dibenamine.

following insulin or formalin injection in animals pretreated with Dibenamine might be explained either (a) by partial blocking of the action of epinephrine on ACTH secretion (according to Long), or (b) as due to the increased amounts of adrenal cortical hormone present in the circulation as a result of the Dibenamine.

SUMMARY

1. Adrenal cortical hormone levels in the adrenal vein blood have been determined in unanesthetized trained dogs, using a modified London cannula. Values of 14 to 50 microgram equivalent of Compound A acetate per ml. of plasma were obtained.

2. Adrenal cortical hormone was demonstrated in the peripheral arterial blood of normal dogs.

3. The levels in peripheral blood rose following injection of ACTH or of epinephrine.

4. Following an "alarming stimulus" (injection of formalin, colchicine, or insulin), the hormone levels in peripheral arterial blood rose. The greatest rise was obtained with insulin and the smallest with colchicine.

5. Injection of the sympathetic blocking drug Dibenamine was followed by a rise in the level of cortical hormone in peripheral blood: Dibenamine therefore acts as an "alarming stimulus."

6. Insulin or formalin, injected into dogs pretreated with Dibenamine, caused a smaller rise in the level of cortical hormone in peripheral blood than was observed following insulin or formalin alone.

REFERENCES

ANDERSON, E. AND HAYMAKER, W.: *Proc. Soc. Exp. Biol. and Med.* **38**: 610. 1938.
CRANDALL, L. A. AND LIPCOMB, A.: *Am. J. Physiol.* **148**: 312. 1947.
DORFMAN, R. I., SHIPLEY, R. A., SCHILLER, S. AND HORWITT, B. N.: *Endocrinol.* **38**: 189. 1946.
LEBLOND, C. P., AND SEGAL, G.: *Cpt. rend. Soc. de Biol.* **128**: 995. 1938.
LONG, C. N. H.: Recent Progress in Hormone Research. **I**: 99. 1947.
LONG, C. N. H., AND FRY, E. G.: *Proc. Soc. Exp. Biol. and Med.* **59**: 67. 1945.
PASCHKIS, K. E., CANTAROW, A., WALKLING, A. A., PEARLMAN, W. H., RAKOFF, A. E., AND BOYLE, D.: *Fed. Proc.* **7**: 90. 1948.
PASCHKIS, K. E., CANTAROW, A. AND BOYLE, D.: *Fed. Proc.* **8**: 123. 1949.
SAYERS, G., AND SAYERS, M. A.: *Rec. Progress in Hormone Res.* **2**: 81. 1948.
SAYERS, G., AND SAYERS, M. A.: *Ann. N. Y. Acad. Sci.* **50**: 522. 1949.
SEIFTER, J., EHRICH, WM. E., BEGANY, A. J., HUDYMA, G. M.: *Fed. Proc.* **8**: 331. 1949.
TEPPERMAN, J., AND BOGARDUS, J. S.: *Endocrinol.* **43**: 448. 1948.
THORN, G. W., AND FORSHAM, P. H.: *Rec. Prog. Hormone Res.* **4**: 229. 1949.
VENNING, E. H., KAZMIN, V. E., AND BELL, J. C.: *Endocrinology* **38**: 79. 1946.
VOGT, M.: *J. Physiol.* **102**: 341. 1943.
VOGT, M.: *Exp. Med. and Surg.* **5**: 279. 1947.
ZECKWER, J. T.: *Am. J. Physiol.* **152**: 267. 1948.

AN EVALUATION OF AFFERENT NERVOUS IMPULSES IN THE ADRENAL CORTICAL RESPONSE TO TRAUMA

MARVIN L. GORDON[1]

Institut de Médecine et de Chirurgie expérimentales, Université de Montreal, Montreal

DIRECT stimulation of a peripheral nerve (Long, 1946), and exposure of an organism to such purely neurotropic stimuli as sound and light (Fortier, 1949, 1950), has been shown to produce an increase in adrenal cortical activity. It is the purpose of this investigation to learn to what extent afferent nervous impulses participate in the increased adrenal cortical activity following trauma (Selye, 1946). The adrenal ascorbic acid concentration was determined, as an index of adrenal cortical activity (Sayers and Sayers, 1948), following local trauma (i.e., fracture and scalding) to innervated and denervated limbs.

Painful stimuli are known to evoke a discharge of epinephrine. Epinephrine has in turn been shown to be capable of stimulating the corticotrophic activity of the pituitary (Vogt, 1944; Long and Fry, 1945), although it is not essential for the increased adrenal cortical activity following nonspecific stress (Recant *et al.*, 1950; Gordon, 1950). Since it was possible that the afferent nervous component of trauma was mediated through the secretion of epinephrine by the adrenal medulla, this possibility was also investigated, and eliminated, by demonstrating that this nervous component was also present in adrenal-demedullated animals.

METHODS

Female piebald rats weighing 140–200 gms, were used. Adrenal demedullations were done by the method described by Evans (1936) 35 to 40 days before the animals were sacrificed.

The left hind leg of all animals, including controls, was denervated by sectioning the sciatic and femoral nerves. The denervation was considered to be complete if there was no reaction (squealing or struggling) by the rats when the tibia or foot was pinched with a forceps. By this criterion about 75% of the rats were felt to be completely denervated, and only these rats were included in the groups in which the denervated leg was traumatized. Occasionally one of these animals reacted to the trauma, and these results were discarded.

Received for publication May 24, 1950.

[1] This work was done during the tenure of a Life Insurance Medical Research Student Fellowship.

The trauma was administered to unanesthetized rats, to the right (intact) hind leg in the innervated groups, and to the left (denervated) hind leg in the denervated groups. Fracture was produced by snapping the tibia between the thumbs and index fingers. The scalded groups were scalded by grasping the foot with a forceps and immersing it in water at 70°C. for 5 seconds for a mild scald and at 85°C. for 10 seconds for a severe scald. The scalded rats were restrained by being first wrapped in a soft towel.

The fractured rats were sacrificed 1 and 2½ hours after fracture (Table 1) and the

TABLE 1. ROLE OF AFFERENT NERVOUS IMPULSES IN THE RESPONSE OF THE ADRENAL CORTEX TO FRACTURE

	No.	Duration (hrs.)	Adrenal ascorbic acid conc. (mg./100 gm.)	Decrease
A. Normal animals				
Denervated controls	7	—	538 ± 12	—
Fracture—innervated leg	7	1	413 ± 22	23%
Fracture—denervated leg	7	1	456 ± 22	15%
Fracture—innervated leg	5	2½	340 ± 11	37%
Fracture—denervated leg	6	2½	482 ± 23	10%
B. Adrenal demedullated animals				
Denervated controls	8	—	487 ± 28	—
Fracture—innervated leg	6	2½	350 ± 21	28%
Fracture—denervated leg	6	2½	427 ± 25	12%

scalded rats were all sacrificed 2½ hours after scalding. The adrenal ascorbic acid concentration was determined by the method of Roe and Kuether (1943).

RESULTS

Fracture

It is seen (Table 1A) that a greater discharge of adrenal ascorbic acid was produced by fracture of an intact leg than by fracture of a denervated leg. This difference was slight and not significant 1 hour after fracture, but quite marked 2½ hours after fracture. At this later period the animals who suffered a fracture of the innervated leg showed a still greater decrease in the adrenal ascorbic acid concentration than at the 1 hour interval, while those animals which suffered a fracture of the denervated leg had an adrenal ascorbic acid concentration which was not significantly lower than that of the non-traumatized controls.

This difference between the denervated and innervated groups at the 2½ hour interval was also demonstrable in adrenal-demedullated animals (Table 1B).

Scald

A mild scald of the innervated foot produced a marked discharge of adrenal ascorbic acid after 2½ hours but only a slight discharge followed scalding of the denervated foot. These findings were also seen in the adrenal-demedullated animals (Table 2). However, a more severe scald of

the denervated foot produced a discharge of adrenal ascorbic acid equal to that produced by a similar scald of the innervated foot (Table 3).

TABLE 2. ROLE OF AFFERENT NERVOUS FACTORS IN THE RESPONSE OF THE NORMAL AND DEMEDULLATED ADRENAL GLAND TO A "MILD" SCALD

	Number animals	Adrenal ascorbic acid conc. (mg./100 gm.)	Decrease
Denervated controls	9	456 ± 25	—
Scald—innervated leg	6	331 ± 19	27%
Scald—denervated leg	8	429 ± 29	6%
Demedullated—denervated controls	7	406 ± 34	—
Demedullated—scald—innervated leg	6	254 ± 13	37%
Demedullated—scald—denervated leg	8	343 ± 14	16%

DISCUSSION

The results suggest that there are two components to the stimulus responsible for the increased activity of the pituitary-adrenal axis following trauma: neural and humoral. In certain types of trauma such as fracture or a mild scald, where there is little tissue destruction, the stress may be considered to be primarily neurotropic, and afferent nervous impulses play a

TABLE 3. ROLE OF AFFERENT NERVOUS IMPULSES IN THE RESPONSE OF THE NORMAL ADRENAL GLAND TO A "SEVERE" SCALD

	Number of animals	Adrenal ascorbic acid conc. (mg./100 gm.)	Decrease
Denervated controls	6	471 ± 17	—
Scald—innervated leg	6	272 ± 13	42%
Scald—denervated leg	6	277 ± 10	41%

major role in the initiation of adrenal cortical activity. On the other hand severe scalds and other forms of trauma, which produce a greater degree of tissue destruction with the resultant release of toxic products, are capable of activating the pituitary-adrenal axis in the absence of nervous impulses. In such instances afferent nervous impulses can be considered to be of minor importance.

In agreement with this concept are the recent observations that the eosinopenia following surgical operations can be delayed by spinal anesthesia for a period equal to the duration of the anesthesia (Roche *et al.*, 1950), and prevented by previous section of the spinal cord (Hume and Wittenstein, 1950). Since there is little tissue destruction in the usual surgical procedure, operative trauma can be considered a primarily neurotropic stress, and thus the increased adrenal activity which normally follows a surgical operation is readily prevented by interrupting the nervous impulses arising at the site of trauma. The stress of handling and blood withdrawal in rats seems to be also dependent upon adequate sensory perception (Recant *et al.*, 1950).

The mechanism by which nervous impulses and humoral agents activate the corticotrophic activity of the anterior pituitary gland is unknown at the present time. However, it is possible that some humoral agents may act directly upon the anterior hypophysis (Selye, 1946), while nervous impulses are probably relayed through the hypothalamus and the hypophyseal portal system of veins (Harris, 1948). Although highly speculative, such an hypothesis would explain why both histamine (Cheng *et al.*, 1949) and exposure to cold (Fortier and Selye, 1949) produce an increased corticotrophic activity in the transplanted pituitary gland while the adrenal cortical response to emotional stress is prevented by hypothalamic lesions (Harris and De Groot, 1950) and by interruption of the nervous and vascular communications between the hypothalamus and the pituitary (Harris, Personal communication).

SUMMARY

The discharge of adrenal ascorbic acid which normally follows a fracture or mild scald of the leg of normal and adrenal-demedullated female piebald rats was diminished if the traumatized leg had been previously denervated. Denervation had no effect on the adrenal ascorbic acid discharge following a more severe scald.

ACKNOWLEDGEMENTS

This investigation was supported by a research grant from the National Institute of Health, U. S. Public Health Service.

REFERENCES

CHENG, C. P., G. SAYERS, L. GOODMAN AND C. SWINEYARD: *Am. J. Physiol.* **159:** 426, 1949.

EVANS, G.: *Am. J. Physiol.* **114:** 297, 1936.

FORTIER, C.: *Proc. Canad. Physiol. Soc.*, 1949; *Rev. Canad. de biol.* **9:** 70, 1950.

FORTIER, C., AND H. SELYE: *Am. J. Physiol.* **159:** 433, 1949.

GORDON, M. L.: *Endocrinology*, 47: 13, 1950.

HARRIS, G. W.: Personal communication.

HARRIS, G. W.: *Physiol. Rev.* **28:** 139, 1948.

HARRIS, G. W., AND J. DE GROOT: *Fed. Proc.* **9:** 57, 1950.

HUME, D. M., AND G. J. WITTENSTEIN: *Proceedings of the First Clinical ACTH Conference.* 607 pp. Philadelphia: Blakiston Co., 1950. p. 134.

LONG, C. N. H.: *Recent Progress in Hormone Research* **1:** 99, 1946.

LONG, C. N. H., AND E. G. FRY: *Proc. Soc. Exper. Biol. & Med.* **59:** 67, 1945.

RECANT, L., D. M. HUME, P. H. FORSHAM, AND G. W. THORN: *J. Clin. Endocrinol.* **10:** 187, 1950.

ROCHE, M., G. W. THORN, AND A. G. HILLS: *New Eng. J. Med.* **242:** 307, 1950.

ROE, J. H., AND C. A. KUETHER: *J. Biol. Chem.* **147:** 399, 1943.

SAYERS, G., AND M. A. SAYERS: *Recent Progress in Hormone Research* **2:** 81, 1948.

SELYE, H.: *J. Clin. Endocrinol.* **6:** 117, 1946.

VOGT, M.: *J. Physiol.* **103:** 317, 1944.

THE EFFECT OF GLUCOSE ON THE ADRENAL RESPONSE TO ACTH IN HYPOPHYSECTOMIZED RATS

P. CONSTANTINIDES, C. FORTIER,* AND F. R. SKELTON

Institut de Médecine et de Chirurgie Expérimentales,
Université de Montréal

INTRODUCTION

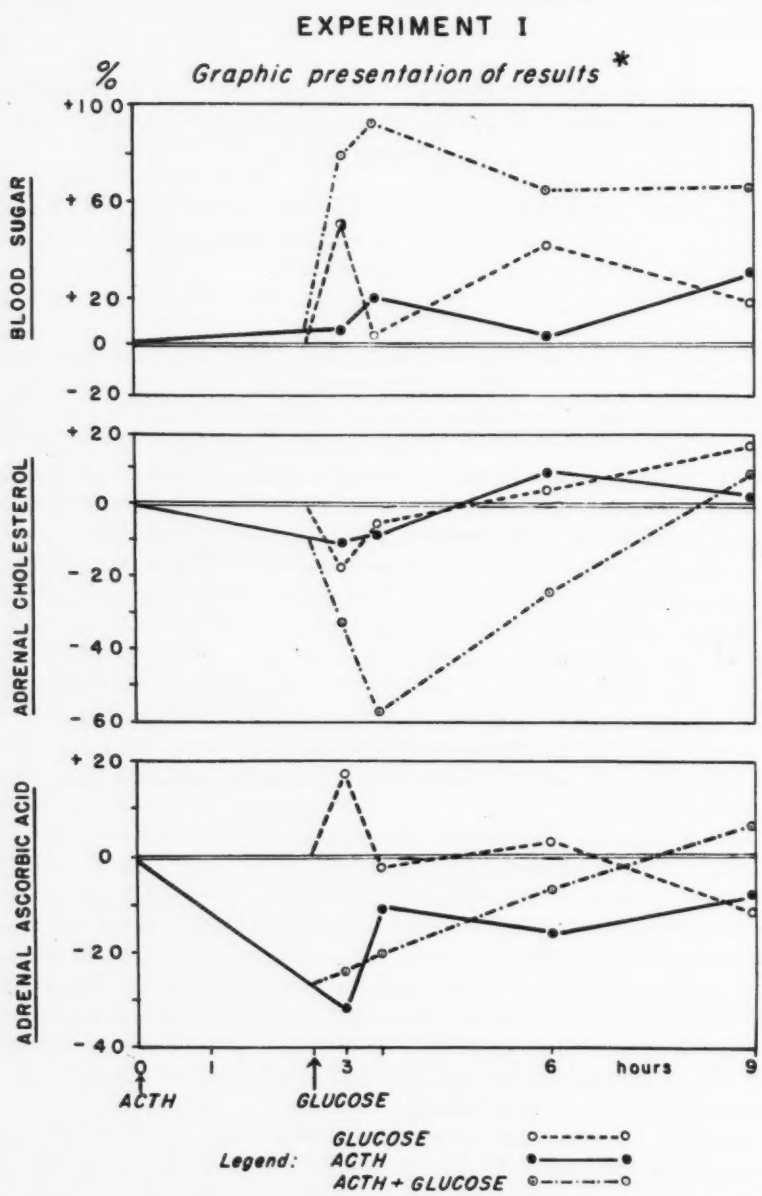
EVER since the pituitary-adrenal system was recognized as the main humoral regulator of reactions to stress, attempts have been made to influence its function by various physiological procedures. The discovery of agents modifying the function of that system, might provide the clinician with effective means of combating diseases resulting from its derangements. The various nutritional and hormonal factors already investigated in that respect have been recently reviewed by one of us (Skelton, 1949).

It has been the common characteristic of "conditioning" research in the past to attempt a modification of the adrenal response to prolonged stress or hormone injections. Only few quantitative studies have been made in non-stressed animals over short periods of time. In the course of such studies over the last years, some evidence has been presented for a rapid action of glucose on the adrenal of resting animals. Abelin (1945) first observed a fall in adrenal cholesterol seven to eight hours following a single oral sugar administration. In line with his first findings, the same author subsequently (Abelin, 1946) noted that glucose increased the adrenal cholesterol response of intact animals to intraperitoneal injections of crude anterior pituitary extracts. Elmadjan, Freeman, and Pincus (1946) reported the occurrence of lymphopenia one to two hours after sugar feeding and recently Jordan (1950) found an eosinopenia under similar conditions, attributing it to ACTH release from the anterior pituitary. Finally, we have confirmed with various hexoses the initial findings of Abelin (Constantinides, unpublished data). These observations all indicate that massive carbohydrate administration will stimulate the release of hormones from the adrenal cortex within a few hours, but they do not point to the nature of the mechanism involved.

In contrast, Steeples and Jensen (1949) reported a rapid rise in adrenal cholesterol 30 minutes after glucose feeding, followed by a fall in the sub-

Received for publication June 5, 1950.

* This work was done during the tenure of a Life Insurance Medical Research fellowship.



* Plotted in the ordinate are the mean values expressed as % deviation from the hypophysectomized control level. The abscissa indicates time intervals in hours from the moment of ACTH injection.

sequent hours. They concluded that hyperglycemia primarily inhibits ACTH production in the anterior pituitary, with a resulting decrease in circulating cortical hormones which would elicit a secondary release of ACTH. Furthermore, one of us (Constantinides, 1950) found that during the first hours of exposure to cold, animals force-fed a high carbohydrate diet discharged less adrenal cholesterol than those maintained on an isocaloric high protein diet.

The experiments reported in this paper were aimed at obtaining a broader physiological evaluation of the effect of glucose on the adrenal and at determining whether this effect is exerted directly on the cortex or indirectly through the pituitary.

MATERIALS AND METHODS

Two experiments were done, employing in both instances male, piebald rats weighing an average of 200 gm. Armour's ACTH was used in the first experiment while Armour's and Li's ACTH were compared in the second.

The following general procedure was adopted: ACTH was injected subcutaneously into hypophysectomized, fasted rats. Two and a half hours later, glucose was administered orally and the animals were killed at several time intervals after the sugar feeding. Hypophysectomized control rats receiving either ACTH or glucose alone were killed at corresponding intervals. In this way, the full action of ACTH on adrenal cholesterol coincided with hyperglycemic blood levels and it was possible to study all events as a function of time. The time schedule for all manipulations was arranged in such a way that all animals were killed 24 hours after the hypophysectomy and samples were removed for the following determinations:

1. Blood glucose (method of Folin, 1929)
2. Adrenal cholesterol (method of Sperry, 1938)
3. Adrenal ascorbic acid (method of Roe and Kuether, 1943)
4. Plasma ascorbic acid (method of Roe and Kuether, 1943)
5. Liver glycogen (method of Good, Kramer and Somogyi, 1933)

Liver glycogen was estimated only in the second experiment. The thymus, the spleen, the iliac lymph nodes and a jejunum loop from every animal of the first experiment were fixed in a mixture of alcohol-formaldehyde-acetic acid and stained with toluidine blue for histological examination.

a) *Experiment I*

All animals were hypophysectomized, subsequently fasted—with free access to tap water—and divided into four groups:

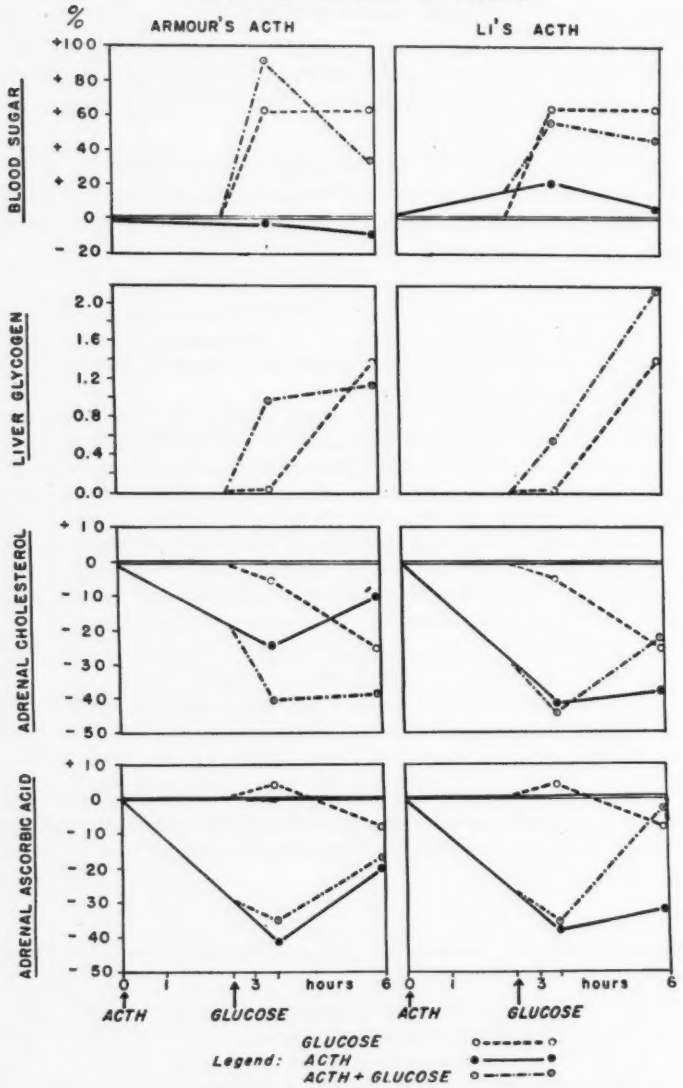
I. Controls receiving no treatment at all
II. Controls receiving glucose only
III. Controls receiving ACTH only
IV. Experimentals receiving ACTH and glucose

Attached to this experiment was a group of four non-operated control rats killed after twenty-four hours of fasting.

The first group, consisting of four rats, was fasted for twenty-four hours and then

EXPERIMENT II

Graphic presentation of results *



* Plotted in the ordinate are the mean values expressed as % deviation from the hypophysectomized control level. The liver glycogen values are expressed as gm. per 100 gm. liver. The abscissa indicates time intervals in hours from the moment of ACTH injection.

killed. The second group received glucose by stomach tube and was subdivided into sub-groups of four rats killed at one half, one, three and a half and six and a half hours after gavage. The third group received ACTH and was subdivided into sub-groups of four rats, killed at intervals of three, three and a half, six and nine hours after injection. The fourth group received ACTH, followed by glucose two and a half hours later and was sub-divided into sub-groups of four rats killed at one half, one, three and a half and six and a half hours after glucose gavage, i.e. three, three and a half, six and nine hours after ACTH injection, respectively.

Diagram number one will facilitate the visualisation of the time schedule for the sub-groups receiving combined treatment. (The arabic figures between the arrows denote time in hours.)

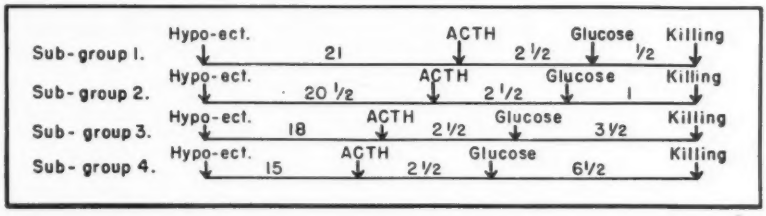


DIAGRAM NUMBER ONE

Omitting ACTH or glucose administration in the above diagram will demonstrate the respective timing of the sub-groups in the glucose or ACTH control groups .

b) *Experiment II*

This was arranged in the same way as experiment I with the following differences:

1. Each sub-group consisted of six rats.
2. The animals receiving glucose, ACTH, or both were killed at only two intervals after the last manipulation. These were 1 and 3½ hours after glucose, i.e. 3½ and 6 hours after ACTH administration.
3. The groups receiving ACTH and ACTH plus glucose were duplicated; two groups were injected with Armour's, the other two with Li's ACTH.
4. No intact controls were included.

Diagram number two illustrates the time schedule for the sub-groups receiving combined treatment in this experiment:

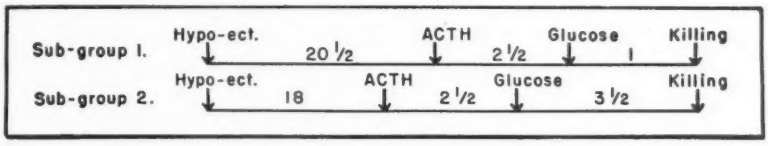


DIAGRAM NUMBER TWO

The following solutions were employed:

Experiment I

1. Armour's ACTH, (lot number 41-L3, equivalent to Armour's standard) administered subcutaneously at a dose of 4.7 mg. dissolved in 0.2 mo. saline.

TABLE 1. EXPERIMENT I (ARMOUR'S ACTH)*
Blood sugar, adrenal cholesterol and ascorbic acid**

Chemical determinations	Blood sugar					Adrenal cholesterol					Adrenal ascorbic acid				
Time intervals after ACTH injection (hours)	0	3	3½	6	9	0	3	3½	6	9	0	3	3½	6	9
Time intervals after glucose gavage (hours)	0	½	1	3½	6½	0	½	1	3½	6½	0	½	1	3½	6½
No treatment	78.0 ±1.5					2.54 ±0.29					498 ±38				
Hypo-etc. +Glucose	95.3 ±2.5	144.0 ±21	101.0 ±7.3	147.0 ±6.1	112.0 ±2.6	3.59 ±0.32	2.93 ±0.34	3.47 ±0.29	3.71 ±1.31	4.18 ±0.91	503 ±35	551 ±13	495 ±28	525 ±11	446 ±15
Hypo-etc. +ACTH	95.3 ±2.5	103.5 ±4.8	115.0 ±15	91.0 ±9.5	122.5 ±22.5	3.59 ±0.32	3.13 ±0.33	3.28 ±0.33	3.88 ±0.77	3.64 ±0.03	503 ±35	350 ±38	452 ±5	425 ±16	460 ±12
Hypo-etc. +ACTH +Glucose	95.3 ±2.5	170.5 ±8.5	184.0 ±23	156.7 ±30.5	156.0 ±17	3.59 ±0.32	2.37 ±0.65	1.50 ±0.32	2.70 ±0.60	3.87 ±0.48	503 ±35	385 ±39	400 ±29	474 ±18	536 ±16

* Armour's ACTH, lot no. 41-L3, 4.7 mg. in 0.2 ml. saline s.c.
** Blood sugar is expressed as mg./100 ml. blood, adrenal cholesterol as mg./100 mg. gland and adrenal ascorbic acid as micrograms/100 mg. gland.

TABLE 2. EXPERIMENT II (ARMOUR'S* AND LI'S ACTH)**
Blood sugar, liver glycogen,*** adrenal cholesterol and ascorbic acid

Chemical determination	Blood sugar			Liver glycogen			Adrenal cholesterol			Adrenal ascorbic acid		
Time intervals after ACTH injection (hours)	0	3½	6	0	3½	6	0	3½	6	0	3½	6
Time intervals after glucose gavage (hours)	0	1	3½	0	1	3½	0	1	3½	0	1	3½
Hypo-etc. +Glucose	86 ±10	139 ±9	139 ±13	0.00	0.04 ±0.03	1.39 ±0.24	3.71 ±0.35	3.52 ±0.31	2.78 ±0.32	642 ±43	670 ±25	593 ±20
Hypo-etc. +ACTH (Armour)	86 ±10	85 ±5	78 ±6	0.00	0.00	0.00	3.71 ±0.35	2.82 ±0.35	3.31 ±0.19	642 ±43	381 ±34	514 ±16
Hypo-etc. +Glucose +ACTH (Armour)	86 ±10	160 ±8	116 ±8	0.00	0.99 ±0.33	1.16 ±0.29	3.71 ±0.35	2.23 ±0.22	2.25 ±0.21	642 ±43	415 ±27	531 ±32
Hypo-ect. +ACTH (Li)	86 ±10	104 ±8	90 ±12	0.00	0.00	0.00	3.71 ±0.35	2.16 ±0.33	2.34 ±0.25	642 ±43	395 ±24	437 ±15
Hypo-etc. +Glucose +ACTH (Li)	86 ±10	133 ±9	125 ±5	0.00	0.57 ±0.32	2.13 ±0.36	3.71 ±0.35	2.07 ±0.25	2.89 ±0.44	642 ±43	411 ±37	625 ±32

* Armour's ACTH, lot no. 77-S, 10 mg. in 0.2 ml. saline, s.c.
** Li's ACTH, lot no. L 1667-G, 4 mg. in 0.2 ml. saline, s.c.
*** Liver glycogen is expressed as gm./100 gm. liver, the other values are expressed as in Table 1.

Experiment II

1. Armour's ACTH, lot number 77-S. (This lot had a potency equivalent to 40% of Armour's standard, the latter being equivalent gram for gram to Li's ACTH). 10 mg. of this ACTH, dissolved in 0.2 ml. saline were administered sub-cutaneously.
2. Li's ACTH, electrophoretically pure, lot number L 1667G. As it was desired to compare the two ACTH preparations in biologically equivalent amounts, 4 mg. of this ACTH, dissolved in 0.2 ml. saline were given sub-cutaneously.

A 50% glucose solution was used in both experiments and administered by stomach tube as a dosage of 2 ml. (i.e. 1 gram) per animal.

The animals of both experiments were thoroughly checked for completeness of hypophysectomy and the few incompletely operated rats were eliminated. However, as both experiments were started with an excess of animals, elimination did not diminish the number of animals per group beyond the figures stated above.

RESULTS

(See tables and figures)

a) *Blood Sugar*

It will be noted that at the end of a 24 hour fasting period the hypophysectomized controls had higher blood sugar values than the intact controls.

In both experiments, glucose alone caused a rapid hyperglycemia which was returning towards normal six and a half hours after its administration.

Neither Armour's nor Li's ACTH showed any effect on the blood sugar, when given alone, but Armour's ACTH increased the immediate alimentary hyperglycemia when given with glucose, while Li's ACTH showed no such effect.

b) *Liver Glycogen*

Appreciable glycogen deposition from glucose can be seen three and a half hours following glucose feeding while neither of the ACTH preparations led to any glycogen formation up to six hours after injection.

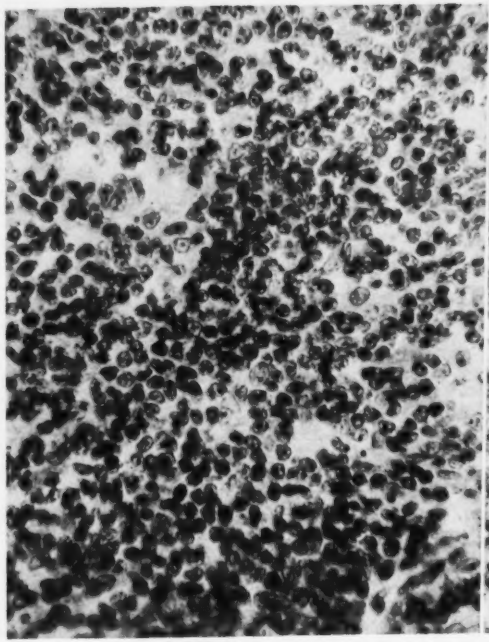
The same amount of glucose, preceded by Li's ACTH gave rise to an earlier and greater glycogen deposition than that resulting from glucose alone. The same effect was obtained with Armour's ACTH, but it was limited to the first hour after sugar feeding.

c) *Adrenal Cholesterol*

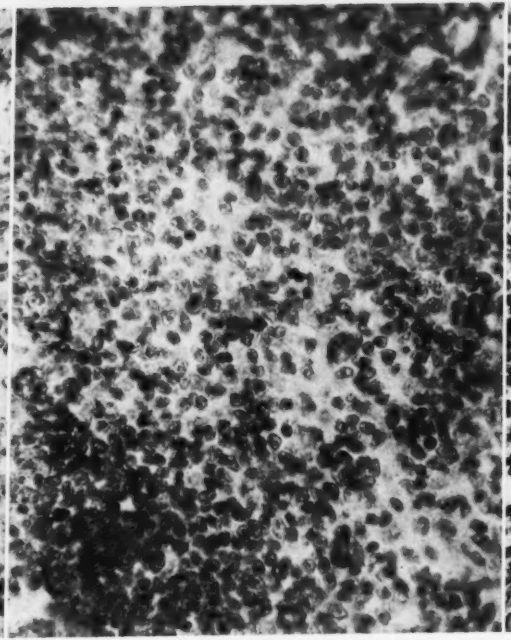
Hypophysectomy causes the usual increase in the cholesterol content of the adrenals.

In both experiments only slight changes were elicited by glucose or Armour's ACTH when each agent was given alone. When Armour's ACTH was followed by glucose, however, a striking discharge of adrenal cholesterol occurred.

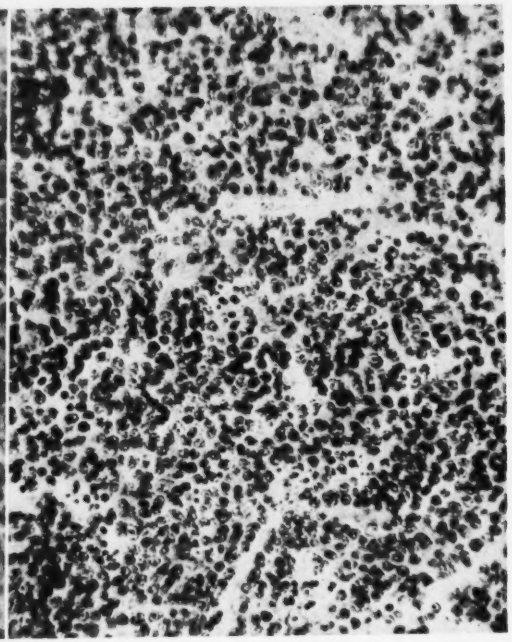
PLATE I



Microphotograph No. 1: ×450. Thymus of a hypophysectomized untreated rat.



Microphotograph No. 2: ×450. Thymus of hypophysectomized rat three and a half hours after the s.c. injection of 4.7 mg. Armour's ACTH.



Microphotograph No. 3: ×450. Thymus of a hypophysectomized rat three and a half hours after the s.c. injection of 4.7 mg. Armour's ACTH and one hour after oral sugar administration.

Li's ACTH produced a significant fall of adrenal cholesterol, but the addition of glucose did not augment this effect.

d) *Adrenal Ascorbic Acid*

Glucose had no appreciable influence on this adrenal constituent.

The two ACTH preparations were equally active in discharging adrenal ascorbic acid.

Glucose supplementation accelerated the return to normal levels of this adrenal metabolite, the effect being particularly visible nine hours after the injection of Armour's and six hours after the injection of Li's ACTH.

e) *Plasma Ascorbic Acid*

Detailed quantitative data on the plasma ascorbic acid will be presented in a separate publication as they form an integral part of a systematic investigation (Skelton and Fortier, in press).

In the present experiments, hypophysectomy caused a fall in the level of plasma ascorbic acid. Armour's ACTH, as well as glucose elicited a rise above the hypophysectomized level while both agents given together occasioned a greater elevation than either alone. Li's ACTH did not cause any significant movement of the plasma ascorbic acid level when given alone, but it augmented the response of this metabolite to glucose.

f) *Lymphatic Caryoclasia*

Histological examination of the lymphatic organs revealed no caryoclasia in the thymus, spleen, and iliac lymphnodes of the intact and hypophysectomized untreated controls (Plate I, microphotograph 1). Only traces of caryoclasia were visible in the patches of Payer of these groups.

Glucose administration did not change the picture obtained in the untreated controls, while ACTH produced a very mild degree of caryopycnosis between three and six hours after injection, (Plate I, microphotograph 2), the effect having disappeared at nine hours.

Combining the ACTH with glucose resulted in a prompt augmentation and prolongation of the effect obtained with ACTH alone. As can be seen in microphotograph 3, Plate I, frank caryoclasia appeared in this group.

DISCUSSION AND CONCLUSIONS

Armour's ACTH will be discussed first as the modification of its effects by glucose appears to reconstruct more closely the actions obtained by hexose administration in intact animals. Since the intensity of lymphatic caryoclasia paralleled the adrenal cholesterol discharge, it seems reasonable to regard the latter as an index of actual adrenocortical hormone release.

The first question that arises is the mechanism through which oral sugar administration increases the adrenal response to this ACTH.

Various possibilities have to be considered. In the first place, glucose might act by simply raising the vitality of the recently operated animals. It would thus act indirectly, through improvement of ACTH absorption as well as through an elevation of all vital processes.

This hypothesis is unlikely in view of the fact that the hypophysectomized untreated controls showed higher blood sugar values than the intact untreated controls and furthermore, if the primary action of glucose consisted in raising all vital responses, the adrenal cholesterol reaction to Li's ACTH should have been increased in the same way as that to Armour's ACTH—which was not the case.

It might yet be considered that the hyperglycemia itself accelerates the synthesis and release of cortical steroids through some process or other, e.g. by providing precursor materials (Bloch and Rittenberg, 1942, Reichstein and Shoppee, 1943), or available energy. Experiments similarly designed as those of the present investigation, but replacing glucose by adrenaline or insulin injections should easily test this hypothesis. Such studies are under way.

Most of the facts can be explained more easily if it is assumed that the carbohydrate excess calls on certain adrenocortical hormones for its disposal in the form of glycogen.

Considerable evidence exists for the control of glycogenesis from glucose by the adrenal cortex. Among the relevant in vivo and in vitro investigations we may mention the findings of Britton and Silvette (1932), Dambrosi *et al.* (1933), Fernandez *et al.* (1934), Russel (1938, 1939), Seckel (1940), and Corey and Britton (1941).

Since glycogen formation from glucose proceeded slowly in the absence of ACTH, but rapidly in its presence, it appears that glycogenic adrenal factors were secreted in response to this hormone. Furthermore, as ACTH elicited a much greater adrenal steroid discharge when given with sugar than when given alone, it seems possible that the degree of adrenal mobilisation in response to ACTH depends on the metabolic necessity for its occurrence.

Attention may be drawn at this point to the dissociation between ascorbic acid and cholesterol movements in the adrenals of animals receiving combined treatment.

It must be kept in mind that these experiments were principally designed to examine the cholesterol response to ACTH. It was for this reason that the earliest adrenal sample was taken three hours after ACTH injection, i.e. at a moment just preceding its maximum effect on the cholesterol, but past its maximum effect on the ascorbic acid reaction.

Thus, while our data illustrate the influence of glucose on beginning, full development and end of the cholesterol reaction, only the returning

part of the ascorbic acid curve was in focus. Nevertheless, it can be seen that the two reactions are conditioned in opposite directions, the cholesterol repletion being retarded, the ascrobic acid repletion being accelerated.

We have observed a similar dissociation in acute experiments where glucose was given to intact animals and endogenous ACTH production was elicited by cold (Fortier, Skelton and Constantinides, unpublished data). An explanation of this interesting occurrence will have to await an understanding of the exact role played by adrenal ascorbic acid in the production and discharge of cortical steroids.

We may, now, turn our attention to some discrepancies in the action of Armour's and Li's ACTH. The two preparations had similar effects as regards adrenal ascorbic acid, plasma ascorbic acid and liver glycogen but different effects as regards adrenal cholesterol and blood sugar.

There is little doubt about the conditioning action of glucose on Armour's ACTH since it was obtained twice—the second time significantly. Our failure to augment the cholesterol effect of Li's ACTH with sugar does not necessarily prove a qualitative difference between the two hormone preparations. It must be emphasized that both ACTH types were given in amounts calculated to be biologically equivalent to each other, and in fact, they produced an identical discharge of adrenal ascorbic acid in the same period of time, i.e. three and a half hours after injection. Nevertheless, they displayed a different potency with respect to the adrenal cholesterol discharge.

It will be recalled that the maximum cholesterol depletion after Armour's ACTH alone was of the order of 15% in the first and 25% in the second experiment. Only when glucose was added did the effect become marked or significant. In evaluating the weak cholesterol action of Armour's ACTH it must be kept in mind that the dose/body weight ratio used was small, approximately half that used by Long (1947).

By contrast, Li's ACTH produced a significant cholesterol depletion of its own without any further amplification of the effect by glucose. It is conceivable that the cholesterol response to Li's ACTH was maximal or near-maximal, no further increase being possible.

The second discrepancy, i.e. the different action of the two hormone materials on the alimentary hyperglycemia, is more difficult to explain without assuming some qualitative difference between the two preparations. Whether this difference reflects the presence of synergistic principles in Armour's preparation, absent from Li's electrophoretically pure protein, or whether it indicates a partial structural difference of the two ACTH molecules cannot be decided without further investigation.

Correlating our findings with those of the literature, we arrive at the following general conclusions:

A single administration of glucose leads within a few hours to an appreciable release of cortical hormones in the intact animal (Abelin, 1945, Elmadjan, *et al.*, 1947, Jordan 1950, our own unpublished observations). Sugar also augments the adrenal cholesterol reaction to saline anterior pituitary extracts (Abelin, 1946). As this effect can be reproduced by glucose and a small dose of Armour's ACTH in the hypophysectomized rat, but not by either agent alone, it must be due largely to an increase of the adrenal responsiveness to ACTH.

If we assume that lymphatic caryoclasia is an index of protein catabolism we would expect glucose to decrease both processes, on the basis of Engel's work (1949). Our histological observations pointed to the contrary. It is suggested for this reason that events at the last level of the pituitary-adrenal-lymphatic tissue chain may be the net results of events at all three levels of this chain. It is quite possible that under our experimental conditions glucose increased the release of cortical steroids more than it counteracted their protein catabolic effect in the body periphery.

The data of the present investigation, along with those of Abelin, 1945, Elmadjan, *et al.*, 1946, and Jordan 1950, appear to be at variance with the results obtained when intact animals gavaged a high-carbohydrate diet are exposed to cold (Constantinides, 1950). It should be kept in mind, however, that in the experiments reported here, hypophysectomized rats received a single sugar feeding while under the conditions of Constantinides intact animals were pretreated with a high-carbohydrate diet for several days prior to cold exposure. It is relevant in this context that Abelin (1945) reported an abolition of the adrenal cholesterol response to a single sugar gavage, if the animals had been kept on a high-carbohydrate regime several days previously.

SUMMARY

It has been found in hypophysectomized rats that:

1. Both Armour's and Li's ACTH increased the glycogen formation from glucose in the liver, while neither ACTH caused any glycogen formation when given alone up to six hours after injection.

2. Neither Armour's nor Li's ACTH had any significant effect on the blood sugar when given alone, but Armour's ACTH increased the alimentary hyperglycemia produced by oral glucose administration.

3. Armour's ACTH produced a slight adrenal cholesterol discharge which was considerably augmented by the addition of glucose. Glucose alone did not affect the adrenal cholesterol concentration. At the dosage level used, Li's ACTH produced a maximal adrenal cholesterol discharge which was not influenced by glucose.

4. Armour's ACTH had only a slight and transitory action on the lymphatic tissues when given alone. The addition of glucose considerably augmented and prolonged this effect. Glucose alone did not affect the lymphatic organs.

5. Glucose accelerated the adrenal ascorbic acid repletion following injection of either Armour's or Li's ACTH. Glucose alone had no significant effect on the adrenal ascorbic acid concentration.

6. It is concluded that the administration of glucose augments the adrenal response to ACTH. Various possible mechanisms and implications of this effect are discussed.

ACKNOWLEDGMENTS

This investigation was supported by a research grant from the National Cancer Institute, United States Public Health Service. The authors wish to thank the Armour Company and Dr. C. H. Li for the kind supply of ACTH.

REFERENCES

ABELIN, I.: *Helv. Physiol. Pharmacol.* Acta **3**: 71. 1945.
ABELIN, I., AND G. BRACHER: *Helv. Physiol. Pharmacol.* Acta **4**: 383. 1946.
BLOCH, K., AND D. RITTENBERG: *J. Biol. Chem.* **143**: 297. 1942.
BLOCH, K., AND D. RITTENBERG: *J. Biol. Chem.* **145**: 625. 1942.
BRITTON, S. W., AND H. SILVETTE: *Am. J. Physiol.* **100**: 701. 1932.
CONSTANTINIDES, P.: *Fed. . Proc.* **9**: 25. 1950.
COREY, E. L., AND S. W. BRITTON: *Am. J. Physiol.* **131**: 783. 1941.
DAMBROSI, R. G., L. F. LELOIR, AND A. NOVELLI: *Compt. Rend. Soc. Biol.* **114**: 1219. 1933.
ELMADJAN, F., H. FREEMAN, AND G. PINCUS: *Endocrinol.* **39**: 293. 1946.
ENGEL, F. L., S. SCHILLER, AND E. I. PENTZ: *Endocrinol.* **44**: 458. 1949.
FERNANDEZ, R., V. G. FOGLIA, L. F. LELOIR, AND A. NOVELLI: *Compt. Rend. Soc.Biol.* **115**: 334. 1934.
FOLIN, O.: *J. Biol. Chem.* **82**: 83, 1929.
FORTIER, C., AND F. SKELTON: *Proc. Canad. Physiol. Soc.* **13**: 13. Oct. 1949.
GOOD, C. A., H. KRAMER, AND M. SOMOGYI: *J. Biol. Chem.* **100**: 485. 1933.
JORDAN, P. H.: *Proc. Soc. Exp. Biol. & Med.* **73**: 243. 1950.
LONG, C. N. H.: Recent Progress in Hormone Research **1**: 99. 1947.
REICHSTEIN, T., AND C. W. SHOPPEE: Vitamins and Hormones **1**: 346. 1943.
ROE, J. H., AND C. A. KUETHER: *J. Biol. Chem.* **147**: 399. 1943.
RUSSEL, J. A.: *Am. J. Physiol.* **121**: 755. 1938.
RUSSEL, J. A.: *Am. J. Physiol.* **128**: 552. 1939.
SECKEL, H. P. G.: *Endocrinol.* **26**: 97. 1940.
SKELTON, F.: Ann. N. Y. Acad. Sciences—Symposium of the adrenal cortex January, 1950—(in press).
SKELTON, F., AND C. FORTIER: In press.
SPERRY, W. M.: *Am. J. Clin. Path.* **2**: 91. 1938.
STEEPLES, G. L., AND H. JENSEN: *Am. J. Physiol.* **157**: 418. 1949.

INFLUENCE OF HYPOPHYSECTOMY ON THE HEMOPOIETIC RESPONSE OF RATS TO LOWERED BAROMETRIC PRESSURES[1]

WILLIAM M. FEIGIN AND ALBERT S. GORDON

From the Department of Biology, Washington Square College of Arts and Sciences, New York University, New York, New York

RECENT experiments have established a relation between the pituitary and hemopoiesis (reviewed by Gordon and Charipper, 1947). Thus anemia, reticulopenia and bone marrow hypoplasia have now become recognized as a feature of the post-hypophysectomy state in the rat (Vollmer, Gordon, Levenstein and Charipper, 1939; Crafts, 1941). These blood deficiencies, moreover, can be overcome by anterior pituitary and other hormones especially those of thyroidal or androgenic nature (Vollmer and Gordon, 1941; Vollmer, Gordon and Charipper, 1942; Crafts, 1946 a, b).

An important research linking the pituitary to hemopoiesis is that of Stewart, Greep and Meyer (1935) who observed that hypophysectomized rats exposed to lowered barometric pressures 25 to 32 days following the operation, failed to respond with the characteristic increases in hemoglobin and red cell counts seen in similarly exposed unoperated rats. On the basis of these as well as other results, the suggestion was made (Gordon and Charipper, 1947) that anoxia, the "normal erythrocytogenic stimulus," may produce its effects on blood formation through the mediation of the endocrine system. It was felt, however, that the experiments of Stewart, Greep and Meyer (1935), by employing only one level of barometric pressure (i.e. 411 mm. Hg), did not sufficiently explore the potential hemopoietic capacities of the hypophysectomized animal. The present research is concerned with a repetition of the experiments of these workers and, in addition, will describe the effects of a lower level of barometric pressure upon the peripheral blood and bone marrow of the hypophysectomized rat.

MATERIALS AND METHODS

Male rats of the Sprague-Dawley strain weighing 130–160 gm. at the start of the experiment were used. Hypophysectomy was performed by the parapharyngeal approach and the data include records of only completely hypophysectomized animals. The rats were kept in a constant temperature room and were fed a well balanced laboratory ration

Received for publication June 6, 1950.

[1] Aided by a grant from the Dazian Foundation for Medical Research.

frequently supplemented by fresh vegetables. The hypophysectomized animals were given further supplements of meat and evaporated milk.

The rats were divided into 6 Groups. The first Group consisted of 20 hypophysectomized animals not exposed to simulated altitudes. Twenty unoperated controls comprised the second Group. In the third Group were 4 hypophysectomized animals subjected to 422 mm. Hg, corresponding to a simulated altitude of 16,000 feet, in a specially constructed low pressure chamber, for periods of 6 hours per day for 14 consecutive days. Four unoperated controls exposed simultaneously with Group 3 comprised Group 4. Group 5 consisted of 10 hypophysectomized rats subjected to pressures of 321 mm. Hg, equivalent to a simulated altitude of 22,000 feet, for periods of 6 hours per day for 14 days. Group 6 contained 10 unoperated controls exposed to the same pressures. Exposure to the reduced pressures was begun at 2 months following hypophysectomy, at which time the red cell counts level off to 60–70% that of normal (Vollmer, Gordon, Levenstein and Charipper, 1939).

In Groups 1 and 2, peripheral blood cell determinations were made periodically throughout the course of the experiment. In Groups 3, 4, 5 and 6, total red and white blood cell numbers, hemoglobin values and reticulocyte percentages were determined just before the first, the seventh, and after the fourteenth day of subjection to lowered pressures.

The total erythrocyte counts were carried out in duplicate using tail blood. Counts were made on a Spencer hemacytometer and were required to agree with ±4%. Total leucocyte numbers were ascertained in a similar manner, all counts being required to check within ±10%.

Reticulocytes were counted in wet smears from blood diluted with 2% sodium citrate solution and stained with brilliant cresyl blue. One thousand red blood cells were examined and the reticulocytes expressed as a percentage.

Hemoglobin determinations were made with a Klett photoelectric colorimeter, using a Klett standard for comparison considering 14.5 gm. per 100 cc. as 100% hemoglobin.

The bone marrows were obtained from right femurs at the termination of the experiment. The bones were cut at both proximal and distal ends, then cracked sufficiently to permit removal of the marrows. These were fixed in Bouin's solution, sectioned in paraffin, and stained in Harris' hematoxylin and eosin.

RESULTS

The statistical analysis indicates that the hypophysectomized rats subjected to 411 mm. Hg. for 7 and 14 days (Group 3) displayed insignificant changes in red cell count and hemoglobin concentrations (Table 1). The mean leucocyte counts of the rats of this Group before exposure was 13,600/cu.mm. and at the termination of 14 days of exposure had reached 14,900/cu.mm., a statistically insignificant rise. The reticulocytes also exhibited an insignificant change from a mean of 0.2% to 0.4% after the 14 day exposure period.

Table 1 reveals that the control rats (Group 4) responded positively to pressures of 411 mm. Hg with increased numbers of red blood cells and hemoglobin values. Leucocyte changes were insignificant. Significant reticulocyte rises were seen in the 7 day- but not in the 14 day-exposed animals.

In contrast to the results obtained at 411 mm. Hg, subjection of hypophysectomized rats to pressures of 321 mm. Hg (Group 5) resulted in significant increases in red cell numbers and hemoglobin concentrations. The response was as great as that exhibited by the unoperated controls (Group 6) exposed to the same intensity of pressure (Table 1). Leucocytes were affected insignificantly in Groups 5 and 6. Reticulocyte counts were higher in both the control and hypophysectomized Groups after 14 days of exposure.

TABLE 1. MEAN ERYTHROCYTE COUNTS (MILL./CU. MM.) AND HEMOGLOBIN PERCENTAGES (±STANDARD ERRORS) OF HYPOPHYSECTOMIZED AND CONTROL RATS SUBJECTED TO SIMULATED ALTITUDES

Group	0 day	7th day	% change	14th day	% change	Critical Ratio[1]
3 RBC	6.2±0.1	5.8±0.4	−7.7	7.1±0.5	14.2	1.5
Hb	90.5±3.0	93.0±6.5	2.8	94.6±8.0	4.5	0.5
4 RBC	8.5±0.6	10.1±0.6	19.6	10.0±0.3	18.4	2.3
Hb	129.0±2.5	147.0±2.2	14.9	168.2±3.7	30.4	8.8
5 RBC	5.8±0.2	7.2±0.3	24.2	8.1±0.3	39.6	6.7
Hb	79.5±3.0	98.0±3.7	23.3	100.5±5.5	26.5	3.9
6 RBC	8.9±0.2	10.7±0.4	19.2	11.6±0.2	28.2	9.8
Hb	113.9±1.6	143.8±2.9	26.4	142.9±4.2	26.0	6.3

[1] Critical ratio $= \frac{\text{Mean}_2\ (14\text{th day}) - \text{Mean}_1\ (1\text{st day})}{\sqrt{(\text{S.E.}_2)^2 + (\text{S.E.}_1)^2}}$

If this value is greater than 2, the difference is probably significant (Davenport & Ekas, 1937).

The bone marrows of the hypophysectomized rats not exposed to lowered pressures (Group 1) showed the typical, and already reported hypoplasia (Fig. 1). The marrows of the hypophysectomized rats sub-

EXPLANATION OF FIGURES

All figures are photomicrographs of bone marrow sections magnified 460×.

FIG. 1. Untreated hypophysectomized rat, 2 months following operation. Note marked degree of vacuolization and reduction in total marrow mass.

FIG. 2. Hypophysectomized rat subjected to 411 mm. Hg, 6 hours daily, for 14 days. No significant alterations in the post-hypophysectomy picture are visible (cf. Fig. 1).

FIG. 3. Hypophysectomized rat subjected to 321 mm. Hg, 6 hours daily for 14 days. Stimulation of erythrogenic activity and reduction in degree of vacuolization are apparent (cf. Fig. 1).

FIG. 4. Unoperated rat, subjected to 411 mm. Hg, 6 hours daily, for 14 days. The marrow is hyperplastic and displays increased numbers of actively proliferating erythrogenic nests.

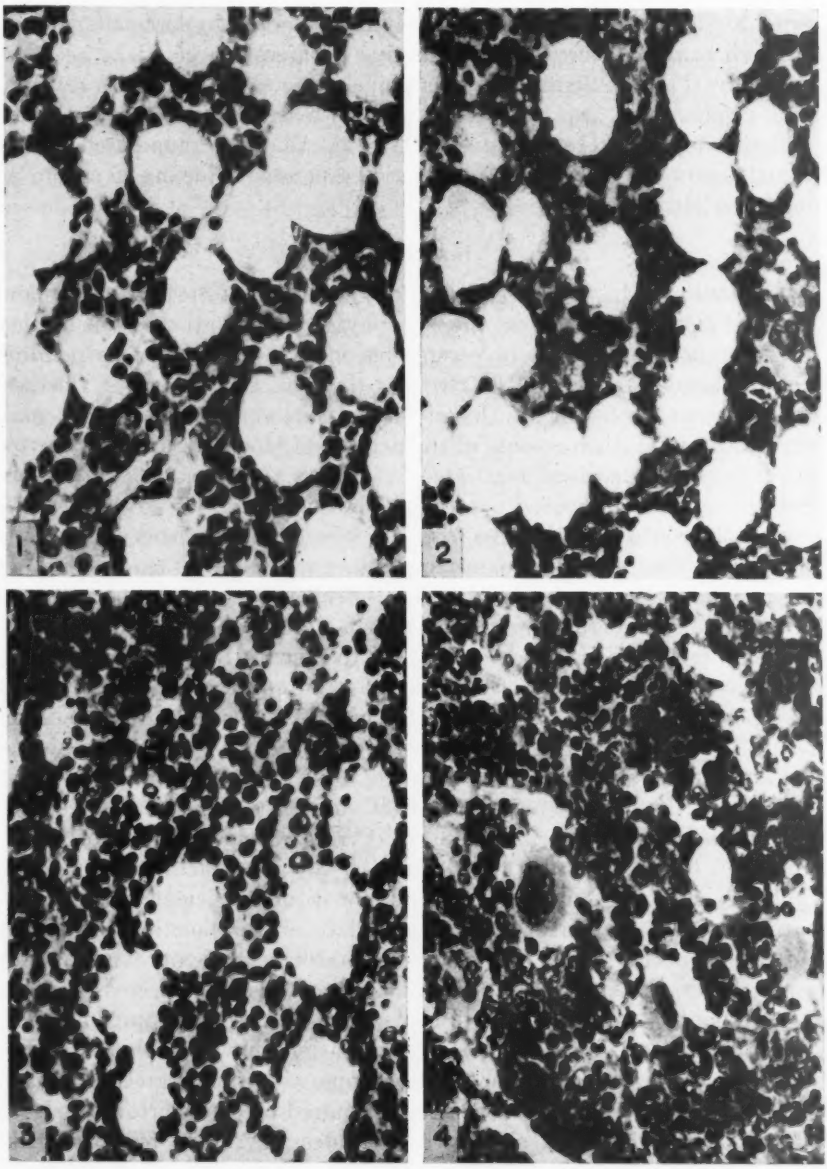


PLATE I

jected to 411 mm. Hg (Group 3) presented a generally hypoplastic pattern, although some scattered areas of active proliferation could be seen occasionally (Fig. 2). Significant repair, including signs of intense erythrogenic proliferation, was visible in the bone marrows of the hypophysectomized rats exposed to 322 mm. Hg (Fig. 3). All of the unoperated control animals showed positive erythropoietic responses following exposure to simulated altitudes (Fig. 4).

DISCUSSION

The results of this study confirm the experiments of Stewart, Greep and Meyer (1935) who found that the hypophysectomized rat does not display an erythropoietic response to lowered barometric pressures corresponding to a simulated altitude of 16,000 feet. At the same time, however, the data of the present work indicate that if the pressures are lowered to 321 mm. Hg. (22,000 feet), the response of the peripheral blood picture and marrow in the pituitary-deprived rats is equivalent to that observed in the unoperated, similarly exposed controls. These latter results do not tend to support the contention that the pituitary is essential for the erythropoietic response of the organism to relatively severe degrees of anoxia but do not preclude the possibility that the effect may have been mediated through some endocrine organs other than the hypophysis.

Several explanations become apparent to account for the refractoriness of the hemopoietic centers of the hypophysectomized animal to pressures corresponding to 16,000 feet altitude. One possibility relates to the fact that the basal metabolic rate of the hypophysectomized animal is reduced (Houssay, 1934) and because of this, its need for oxygen may not be as acute as in the normal animal. This is supported by the findings that thyroidectomy (Streuli, 1918) and antithyroid substances (Gordon, Goldsmith and Charipper, 1944, 1945) increase altitude tolerance in the rat. Thus exposure to 422 mm. Hg. may not constitute sufficient anoxic stress, in the hypophysectomized rat, to stimulate erythropoietic activity; or stating it another way, the reduced blood oxygen concentration corresponding to this pressure is still sufficient to handle the decreased needs of the hypophysectomized animal. It may also be true that compensations, other than those involving red cell formation, have developed in the hypophysectomized rat to enable it to cope with this degree of anoxia.

The finding that the pituitary is not required for the increased red cell production occurring at simulated altitudes of 22,000 feet does not invalidate the possibility that the gland and other members of the endocrine system participate in the hemopoietic responses displayed by the *normal* animal to lowered pressures. The work of Gordon, Tornetta, D'Angelo and Charipper (1943) has indicated that significant effects are

produced by reduced barometric pressures on the structure and function of various endocrine glands. It seems possible that the endocrine organs function like a buffer mechanism, serving to adjust the quality and intensity of erythropoietic response depending upon the demand for oxygen by the body cells. The question as to what extent lowered pressures, in influencing erythropoiesis, operate through the endocrine system, the peripheral cell or directly upon the bone marrow awaits solution.

SUMMARY

Subjection of hypophysectomized rats to reduced pressures of 411 mm. Hg (16,000 feet), for periods up to 14 days, produces no significant effects upon the peripheral red blood cell values, hemoglobin levels or bone marrow histology. Positive responses to these pressures are obtained in the unoperated controls. Pressures of 321 mm. Hg (22,000 feet), however, evoke in the hypophysectomized animal an erythropoietic response of approximately the same magnitude as that displayed by unoperated controls.

It is concluded that although the pituitary is essential for the erythropoietic response to moderately low pressures (411 mm. Hg), a full effect may be produced independently of this gland at more severe levels of anoxia.

REFERENCES

CRAFTS, R. C.: *Endocrinology* **29**: 596. 1941.

CRAFTS, R. C.: *Amer. J. Anat.* **79**: 267. 1946a.

CRAFTS, R. C.: *Endocrinology* **39**: 401. 1946b.

DAVENPORT, C. B., AND M. P. EKAS: *Statistical Methods in Biology, Medicine and Psychology.* John Wiley and Sons, Inc. N. Y. 1946.

GORDON, A. S., AND H. A. CHARIPPER: *Ann. N. Y. Acad. Sci.* **48**: 615, 1947.

GORDON, A. S., E. D. GOLDSMITH AND H. A. CHARIPPER: *Science* **99**: 104. 1944.

GORDON, A. S., E. D. GOLDSMITH AND H. A. CHARIPPER: *Endocrinology* **37**: 223. 1945.

GORDON, A. S., F. J. TORNETTA, S. A. D'ANGELO AND H. A. CHARIPPER: *Endocrinology* **33**: 366. 1943.

HOUSSAY, B. A.: *Endocrinology* **18**: 409. 1934.

STEWART, G. E., R. O. GREEP AND O. O. MEYER: *Proc. Soc. Exp. Biol. and Med.* **33**: 112. 1935.

STREULI, H.: *Biochem. Ztschr.* **87**: 359. 1918.

VOLLMER, E. P., AND A. S. GORDON: *Endocrinology* **29**: 828. 1941.

VOLLMER, E. P., A. S. GORDON AND H. A. CHARIPPER: *Endocrinology* **31**: 619. 1942.

VOLLMER, E. P., A. S. GORDON, I. LEVENSTEIN AND H. A. CHARIPPER: *Endocrinology* 25: 970. 1939.

THE ANDROGENIC ACTIVITY OF OVARIAN TRANSPLANTS TO THE SEMINAL VESICLE OF THE CASTRATED ADULT MALE RAT[1]

SEYMOUR KATSH[2]

From the Department of Biology, New York University Graduate School of Arts and Science, New York, N. Y.

EVIDENCE for the contention that the mammalian ovary elaborates androgenic substance is derived mainly from human ovarian pathologies (e.g. arrhenoblastomata) (Mayer, 1942) and from experiments in which ovaries are transplanted to the ears of castrated male mice (Hill and Gardner, 1936) and rats (Deanesly, 1938). Significantly, Hill (1937a) finds ovaries transplanted to the abdominal cavity of the castrated male mouse do not maintain the accessory reproductive organs, and thus, (1937b) infers that the lower thermal surroundings in the ear site are necessary to the production of androgens by the ovary. Deanesly (1938), however, observes that the androgenic activity of the graft does not depend on temperature, but is associated with the degree of thecal luteinization of the follicles.

Also, the identity of a specific factor, simulating the male hormone, elaborated by the ovary is open to question. Greene *et al.* (1939, 1940) find progesterone possesses androgenicity. Hill, previously (1937a) obtained negative results with this hormone.

The status of the placenta as a source of androgen is not too well established probably because of the limited attention it has received thus far. Cunningham and Kuhn (1941) reported positive results with human placental extracts while Dorfman (1948) obtained negative findings.

In view of this lack of agreement, a more comprehensive reexamination of these points seemed in order. The previous successful employment of the seminal vesicle of the castrated rat as an implantation site (Katsh, Gordon and Charipper, 1948) offered a method of approach. In this procedure, the source material is brought into close proximity with the sensitive epithelium serving as a target organ.

The present work proposes to provide information on the ability of transplants of ovaries from virgin females of different ages up to 120

Received for publication June 26, 1950.

[1] This paper is the main content of a dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Biology, Graduate School of Arts and Science, New York University, N. Y., Feb. 1950.

[2] Present Address: Department of Zoology and Physiology, University of Massachusetts, Amherst, Mass.

days and ovaries and dissected corpora lutea from pregnant rats to stimulate the reproductive accessories of castrated or castrated-adrenalectomized male rats receiving such transplants to the seminal vesicle. For control, the results of the transplanting of pancreas, salivary gland, placenta and fetal membrane tissue obtained from littermates is described. Also included are the results of autografting of thyroid tissue. For purposes of estimating ovarian androgenic potency, autografts and homografts of testicular material were made. The effects of crystalline cholesterol, estrone, progesterone and testosterone propionate when placed in the seminal vesicle are also included. Weight data and histological descriptions of the reproductive accessories and the transplants are presented.

This investigation was sponsored by Professor Harry A. Charipper whose encouragement and advice is hereby acknowledged.

MATERIALS AND METHODS

Six series of a closely inbred strain of adult male rats weighing 170–210 gms. were used. A total of 218 animals were studied. All were fed a balanced mixed diet including fresh water and lettuce.

Table I details animal dispersion into series and groups, giving the number per group

TABLE 1. PLAN OF THE EXPERIMENTS

Series	Group	Number of animals	Mean initial weight and range (gm.)	Mean implant weight and range (mg.)
I	Normal	10	188.5 (179–222)	—
II	Castrate Controls	10	189.8 (175–220)	—
III	Control Implants			
	A. Pancreatic	5	173.0 (170–175)	12.4 (10–15)
	B. Salivary	5	175.2 (170–185)	13.4 (10–16)
	C. Thyroid	10	186.0 (178–200)	6.6 (6–7)
	D. Fetal Membranes	8	177.1 (170–195)	9.6 (6–15)
	E. Placenta	10	182.8 (170–210)	10.9 (6–18)
IV	Testis Implants			
	A. Adult	9	178.8 (170–200)	2.5 (2–3)
	B. 15-day	14	176.9 (170–200)	11.6 (4–30)
V	Ovarian Implants			
	A. 15-day	10	178.0 (170–185)	3.4 (3–4)
	B. 30-day	12	177.8 (175–180)	6.3 (6–7)
	C. 60-day*	20	176.5 (170–190)	9.1 (7–14)
	D. 90-day	15	180.9 (176–190)	9.2 (6–17)
	E. 120-day	14	180.8 (170–210)	12.5 (10–17)
	F. Pregnant	17	179.9 (170–205)	10.3 (6–17)
	G. Corpora Lutea	9	187.2 (170–210)	7.1 (6–9)
VI	Steroid Crystals			
	A. Cholesterol	10	178.4 (170–190)	6.4 (2.4–11.6)
	B. Estrone	10	184.2 (177–196)	4.4 (3.2– 6.2)
	C. Progesterone	10	183.0 (170–200)	5.9 (2.5– 9.5)
	D. Testosterone Propionate	10	187.0 (170–200)	6.3 (2.7–10.6)

* Includes ten animals which were also adrenalectomized.

and the mean initial body weight and range, as well as the mean implant weight and range for each group.

Series I consisted of normal intact rats. All animals in Series II to VI were castrated. Animals in Series II received no other treatment and served as castrate controls.

In Series III, implant controls, five subdivisions were made as follows: Groups A and B consisted of animals into which were transplanted pancreatic and salivary material respectively. These tissues were obtained from littermates. In group C, the animals were thyroidectomized and one lobe of the thyroid autografted to the seminal vesicle. The rats in groups D and E received fetal membrane and placental material respectively. These tissues were secured from females in the last five days of gestation.

Series IV is composed of animals which received testis autografts (group A) and transplants of testis obtained from 15-day old rats (group B).

In Series V the animals were subdivided into 7 groups: groups A–E received ovarian material from dated, weighed and marked littermates. As an additional control in the 60-, 90-, and 120-day groups, vaginal smears (Shorr stained) were made to insure employment of ovaries in a known stage of the cycle. Furthermore, ten of the animals in group C were adrenalectomized and maintained on a 1% solution of NaCl. In group F, portions of ovary from pregnant animals were used, while in group G, corpora lutea, dissected from the main ovarian mass (with the aid of a binocular dissecting microscope) were employed. The implant materials used in these last two groups were obtained from females in the last five days of gestation.

In Series VI four groups of animals received steroid crystals in the seminal vesicles, as indicated in Table 1.

The implantation operative procedure, employed throughout, was as follows: under nembutal anesthesia, the host was castrated, the right seminal vesicle exposed, and its surface incised with fine forceps. To prevent coagulation of the exuding secretion, the vesicle was stroked gently with sterile cotton dipped in a 3% lukewarm solution of chlorazene.[3] The material to be implanted was dissected rapidly from the nembutal-anesthetized donor, weighed on a torsion balance to the nearest mg. and inserted into the incised vesicle. The pocket in the vesicle having been found to heal rapidly, no suture was used.

In the 15- and 30-day ovarian groups, the thyroid group, and in five of the 15-day testis implanted animals, one entire gland was implanted. In all others only a weighed portion was used. Immediately subsequent to implantation, a sample of the material used for grafting was placed in fixative.

Quantities of crystalline cholesterol, estrone, progesterone and testosterone propionate were weighed to the nearest tenth of a mg. and implanted according to the manner indicated above. Crystalline cholesterol and estrone suitable for forceps manipulation were obtained by dissolving (with the aid of a hot water bath) a quantity of the steroid in a beaker containing absolute ethanol. The vessel containing the solution was then plunged into an ice bath and the resultant large crystals were dried in an incubator (56–58 C.).

Twenty-five days subsequent to implantation and castration, the host and littermate of the transplant were sacrificed by ether anesthesia. From each male host, there were secured both vesicles with their anterior prostatic lobes, the ventral prostate and pituitary, while from the littermate of the graft both gonads were obtained. These organs were weighed individually on a torsion balance to the nearest mg. and immediately placed in fixative. All tissues were fixed in Bouin's picro-formol solution. Serial sections of the im-

[3] Abbot's Chloramine-T.

planted vesicle were made at 5μ. Staining was done in Galigher's modification of Harris' Hematoxylin counterstained with eoxin and some slides were stained with Masson's acid Fuchsin aniline blue.

The same sacrificial procedure was used in the steroid series, except that to recover any remaining crystals, the implant-vesicle was first weighed and then manipulated under a dissecting microscope. The recovered steroid from each animal was weighed. The estrone and progesterone residues were placed in Klett fluorimetric tubes containing 5 cc. of absolute ethanol. Colorimetric determinations were made for the recovered estrogen and progesterone using the fluorimetric method of Cohen and Bates (1947), and the ultraviolet procedure of Haskins (1949) respectively.[2]

OBSERVATIONS AND RESULTS

1. *Weight Data*

Table 2 presents the weights of the seminal vesicles and prostates for each of the 6 series of animals.

TABLE 2. WEIGHTS OF THE REPRODUCTIVE ACCESSORIES OF NORMAL, TREATED-CONTROL AND IMPLANTED RATS

Series	Group	Mean sacrifice weight and range (gm.)	Mean R.S.V. weight and range (mg.)	Mean L.S.V. weight and range (mg.)	Mean prostate weight and range (mg.)
I	Normals	217.0 (185–260)	201.5 (185–250)	204.6 (185–245)	202.7 (187–230)
II	Castrate Controls	213.4 (190–245)	24.7 (18–27)	23.8 (17–27)	15.9 (10–17)
III	Control Implants				
	A. Pancreas	194.0 (190–200)	21.2 (18–22)	20.8 (17–23)	14.0 (12–15)
	B. Salivary	189.0 (185–200)	25.0 (23–27)	20.4 (18–22)	12.8 (10–15)
	C. Thyroid	211.0 (195–230)	29.1 (26–32)	24.1 (22–25)	14.4 (13–17)
	D. Fetal Membrane	200.3 (180–225)	24.4 (20–30)	22.0 (16–22)	14.6 (12–20)
	E. Placenta	213.4 (192–260)	47.7 (35–67)	24.2 (20–26)	16.3 (13–18)
IV	Testis Implants				
	A. Adult	228.8 (215–245)	53.0 (45–61)	33.0 (31–36)	27.3 (24–30)
	B. 15-day	218.7 (185–260)	109.6 (54–180)	52.0 (28–75)	47.7 (36–68)
V	Ovarian Implants				
	A. 15-day	220.0 (198–250)	146.0 (84–233)	68.9 (42–93)	62.3 (24–75)
	B. 30-day	192.5 (160–255)	93.8 (78–191)	62.1 (48–76)	40.0 (29–72)
	C. 60-day*	200.7 (170–220)	103.1 (70–220)	61.2 (40–85)	54.6 (35–66)
	D. 90-day	201.9 (170–260)	116.8 (59–185)	46.8 (30–68)	48.4 (25–70)
	E. 120-day	203.2 (175–240)	116.5 (80–187)	60.9 (41–80)	52.7 (30–65)
	F. Pregnant	207.6 (180–245)	117.0 (73–233)	66.5 (42–97)	60.3 (32–87)
	G. Corpora Lutea	223.9 (205–255)	108.8 (90–132)	66.9 (50–85)	60.5 (45–76)
VI	Steroid Crystals				
	A. Cholesterol	206.0 (190–225)	26.1 (24–31)	22.3 (17–27)	13.3 (10–16)
	B. Estrone	207.5 (195–215)	28.1 (22–32)	20.0 (16–24)	14.8 (12–16)
	C. Progesterone	202.5 (180–245)	45.1 (26–50)	25.0 (22–28)	19.2 (14–23)
	D. Testosterone Propionate	209.4 (170–265)	400+	203.9 (180–230)	217.6 (130–310)

R.S.V. = right seminal vesicle.
L.S.V. = left seminal vesicle.
* Includes ten animals which were also adrenalectomized.

It is clear from this table that the implantation of testicular and ovarian tissue and testosterone propionate crystals to the right seminal vesicle prevents to a considerable extent the marked regression in the weights of these organs seen in the castrate-control and control-implant series as well as the cholesterol and estrone groups. Seminal vesicles implanted with placental tissue and progesterone crystals are slightly heavier. The accessories of all other treated groups fall in the castrate range. Animals

receiving testis transplants (IV), ovarian grafts (V) and testosterone crystals (VI, D) presented right seminal vesicles which were morphologically similar in most cases to those found in the normal animal. In these groups many of the glands were distended with a slightly yellow, readily coagulated, viscous secretion. Vesicles which received crystalline testosterone were more than twice the normal size and engorged with secretion.

It is also to be noted that the mates of the testis-, ovarian-, and testosterone-implanted vesicles as well as the ventral prostates in these animals are considerably heavier than the corresponding structures in the control animals. The comparison is illustrated in Fig. 1.

2. *Histology of the Transplants*

All grafts were located in the muscularis or submucosa of the seminal vesicles at varying distances from the lumina. Each was demarcated from the host tissue by a connective tissue capsule.

Control Transplants: Pancreatic and salivary grafts were revealed as necrotic, but fetal membrane and placental tissue implants showed good histological differentiation. The thyroid autografts were well maintained and presented colloid-containing-follicles of varying diameters and shape. Also the presence of droplets in the cytoplasm of the follicular epithelial cells suggests functioning glandular tissue (Fig. 6).

Testis Transplants: The cryptorchid condition characterizes the auto-

---

EXPLANATION OF FIGURES

All figures other than Figure 1 are photomicrographs of tissues fixed in Bouin Picro-formol solution and stained with Galigher's modification of Harris' Hematoxylin and counterstained with eosin.

Figure 1 is a photograph of organs fixed in Bouin Picro-formol solution and dehydrated in 70% alcohol.

FIG. 1. *Top Row:* normal seminal vesicles flanking a ventral prostatic lobe of an ovarian implanted animal. *Middle Row:* accessory organs of the castrated rat. *Bottom Row:* large vesicle contains ovarian implant, the mate vesicle is larger than the castrate and the ventral prostate is that of a normal animal.

FIG. 2. Normal seminal vesicle epithelium with a portion of the anterior prostatic lobe (upper left). (×100).

FIG. 3. Normal epithelium of seminal vesicle showing secretion granules. (×740).

FIG. 4. Seminal vesicle of castrated rat showing degenerative changes 25 days after castration. (×100).

FIG. 5. Marked involution of secretory cells in the seminal vesicle 25 days after castration. (×740).

FIG. 6. Seminal vesicle of castrated-thyroidectomized animal with thyroid graft (lower left). Note the castrate type of vesicle epithelium. (×100).

FIG. 7. Seminal vesicle 25 days after castration showing testicular graft. Note good secretory cell maintenance of the seminal vesicle. (×100).

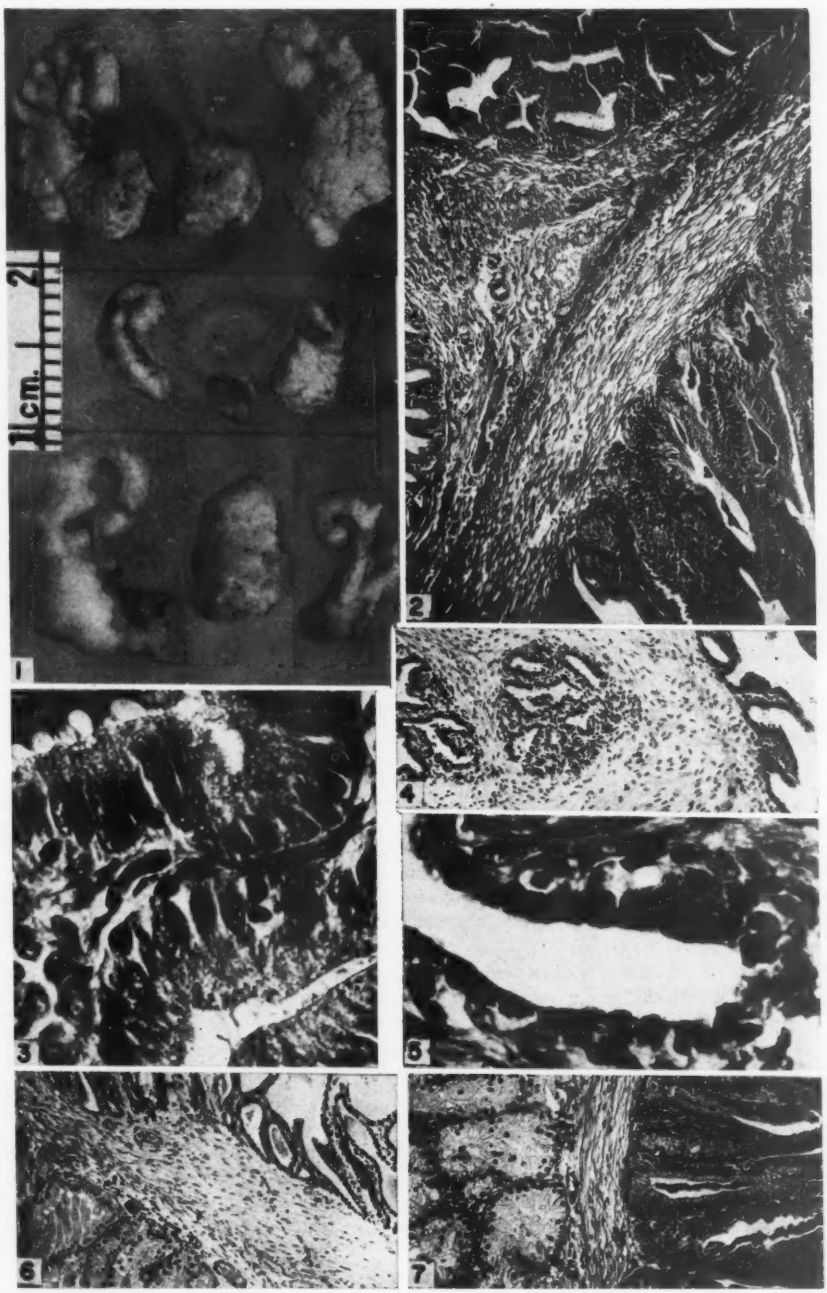


PLATE I

grafts but the 15-day testicular implants do not show so drastic a change. Germinal elements are more common and interstitial tissue is prominent (Fig. 7).

Ovarian Transplants: Although various sizes of grafts were observed, they were normal in most respects. Variation from the normal, established by comparison with fixed control tissues includes some cases in which cystic degenerating follicles are found and others which show a diminution of medullary tissue. Follicles containing ova are found in all stages of growth. The normal complement of the mature follicle is well defined (Fig. 8). Fully formed and developing corpora lutea are seen (Fig. 9 and 10). That some corpora lutea are in the proliferative stages and not persistent is indicated by the presence of mitotic figures and encroachment of luteal cells in cord-like array upon the central blood spot. Comparison with control tissues fixed at the time of transplantation further substantiates this observation since some formative corpora are found in the grafts when none were seen in the control tissues.

Ovarian grafts show no noticeable histological developmental correlation which can be related to the phase of cycle of the donor at the time of implantation. Immature ovarian grafts appear to reach histological maturity more rapidly as comparison with the gonads of control littermates indicates.

Adrenalectomy of 10 castrated males at the time of transplanting 60-day ovaries to their seminal vesicles did not alter the viability or histology of the graft, nor was there an effect on the weight or histology of the acces-

---

EXPLANATION OF FIGURES

FIG. 8. Seminal vesicle of castrated rat with ovarian graft showing complement of large Graafian follicle and tall vesicular epithelium, 25 days. (×100).

FIG. 9. Seminal vesicle with ovarian graft from castrate animal. A portion of a corpus luteum is shown (lower half), note the well-maintained secretory epithelium of vesicle (upper quarter). (×100).

FIG. 10. A portion of a developing corpus luteum of an ovarian-grafted castrate rat. Note vesicular epithelium in upper part of photomicrograph. (×100).

FIG. 11. Ventral prostate of normal animal. (×100).

FIG. 12. Ventral prostate at higher magnification demonstrates light areas (halos) in cells lining the acini. (×740).

FIG. 13. Castrate animal's ventral prostate (25 days). Degenerative changes include small acini with low epithelium and connective tissue overgrowth. (×100).

FIG. 14. Ventral prostate of ovarian implanted animal after 25 days. (Compare with Fig. 11). (×100).

FIG. 15. Higher magnification (same as Fig. 14) demonstrates light areas and granulations of the normal type as seen in the ovarian-grafted animal's ventral prostate (compare with Fig. 12). (×740).

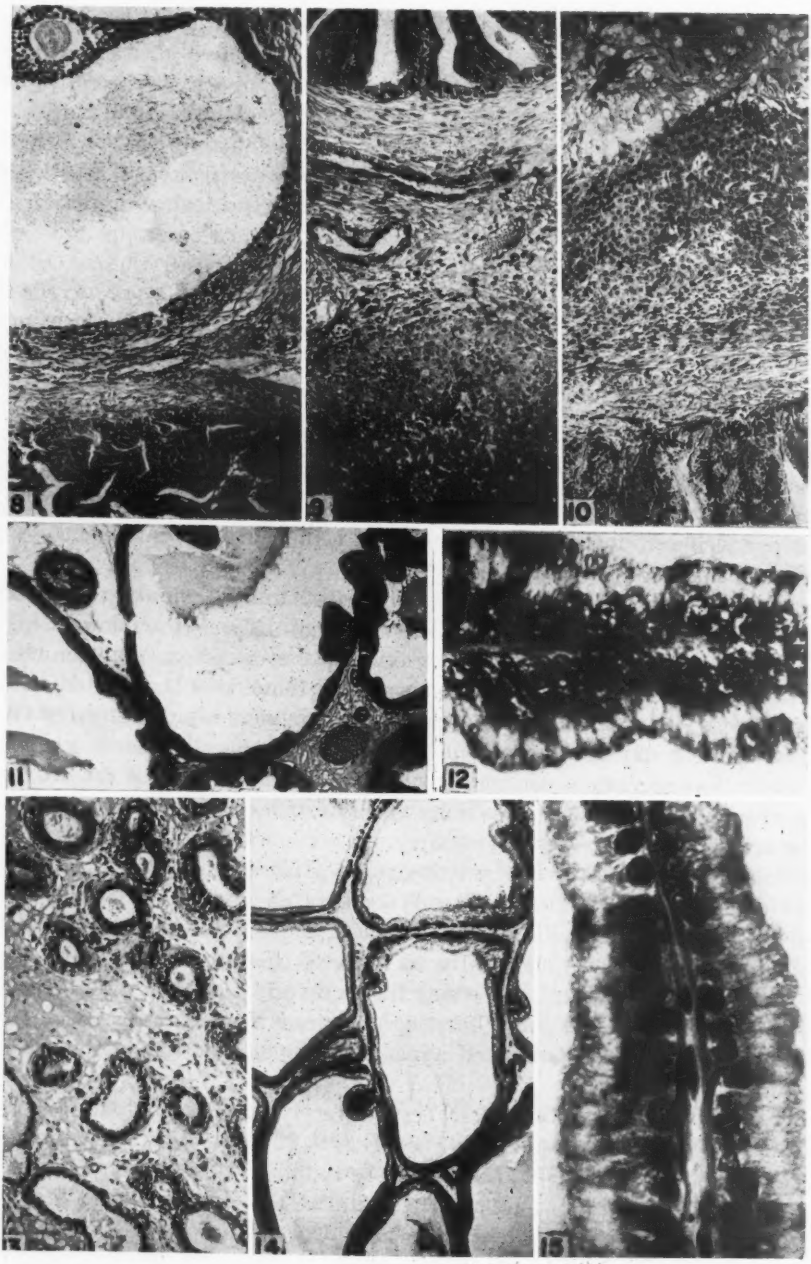


PLATE II

sory organs. Therefore, these data were included with the 10 other 60-day ovarian-grafted animals.

Corpora Lutea Transplants: Although corpora lutea were carefully dissected from ovaries of pregnant rats, histological examination of the graft material which was fixed at the time of transplantation disclosed small quantities of ovarian material adhering to the periphery of the luteal bodies. Apparently these remnants can reconstitute almost an entire ovary for differentiated ovarian grafts were found in this group.

3. *Histology of Seminal Vesicles and Prostate Glands*

Normal and Castrate Control Series: In the normal rat, the secretory epithelium of the seminal vesicle is tall columnar (30–35μ) and the cytoplasm of these cells contains secretory granules (Figs. 2 and 3). Much secretion is present in the lumen. Within 48 hours after castration, the granulations disappear and by the twentieth day after orchidectomy, the epithelium is reduced to a narrow rim of vacuolated cytoplasm. Further all trace of the characteristic secretion has disappeared from the lumen; instead cellular debris is widespread and the entire organ is atrophic (Figs. 4 and 5).

The anterior and ventral lobes of the prostate undergo similar regression. The normal ventral prostatic lobe shows large distended acini with high columnar epithelium and characteristic light areas in the supranuclear zone (Figs. 14 and 15). Within four days after removal of the gonads, these light areas disappear and the epithelium undergoes rapid regression. By the twentieth day involution is indicated by a decrease in acinar size and number, overgrowth of connective tissue obliterating many of the lumina, an absence of secretion and increasing quantities of cellular debris within the acini (Fig. 13).

Control Transplant Series: In this series (III) atrophy of castration is not corrected. For example, Figure 6 shows an apparently actively secreting thyroid graft without stimulation of the vesicle. Also the prostatic lobes of this series are identical with those of the castrate. Similarly, the placental group although possessing higher weights for the grafted vesicle show only the castrate condition. Slight increase in connective tissue plus the weight of the implant itself appears to explain the higher weights of these organs.

Testicular and Ovarian Series: Vesicles receiving testicular and ovarian grafts are markedly stimulated (Figs. 7, 8, 9, 10). The cells are columnar, averaging between 20–30μ, the nuclei are round and basal in position and cytoplasmic granulations are apparent. Unlike the castrate organ, desquamated cells and cellular debris are rare. On the other hand, as in the normals, a colloid-like eosinophilic secretion appears in the lumina. The

most highly activated epithelium is found in proximity to the graft. Some ovarian-bearing vesicles show connective tissue hypertrophy which is presumed to have been effected by estrogenic secretion by the graft.

The contralateral vesicles in these animals reveal a considerably greater degree of histological activity than do the vesicles in any of the control groups. This is in accord with the weight data for these organs.

Also the ventral prostatic lobes in these animals show signs of activation. In some instances the presence of large distended acini and tall epithelium with characteristic light areas mark the gland so that it is indistinguishable from the normal (compare Figs. 11, 12 with 14, 15).

Steroid Series: Animals receiving quantities of cholesterol, estrone and progesterone by implantation of the crystalline material to the seminal vesicles display reproductive accessories which are typically castrate. Estrone does not produce hypertrophy of the connective tissue or the musculature, but some stratification of the epithelium is observed. In the progesterone group, the higher weights of the treated vesicles appear to be due to an increase in connective tissue together with a growth of the musculature.

Table 3 gives the amount of estrone and progesterone administered to each animal (by way of insertion into the seminal vesicle) as well as the amount recovered as determined colorimetrically. This table indicates that some absorption of estrogen and progesterone occurs. Approximately the same quantities of cholesterol and testosterone propionate were used. While residues of cholesterol, estrone and progesterone were recovered from each of the animals so treated, only one of the vesicles implanted with testosterone was found to contain a residue.

In accord with the weight data, testosterone therapy restores the accessories to the normal condition; microscopic examination of these organs shows a condition which compares well with that found in the normal rat except that the vesicle receiving testosterone crystals was considerably larger than normal. Histologically, this condition was reflected in slight growth of the muscularis. The degree of secretory activity, as appraised by the cytoplasmic granulations, is more intense, and the quantity of secretion expressible from these implant-vesicles (before weighing) is greater than that of the normal gland.

DISCUSSION

Primary consideration in these experiments was directed to the ovarian transplants and their ability to stimulate the castrated male's accessory organs, since it seemed pertinent to determine if lower temperature, as in the ear site (Hill, 1937a), is indispensable to androgenic production by an ovarian graft. While the present work does not preclude an enhancing

effect of cooler temperature, it is clearly demonstrated that androgen production proceeds apace in an abdominal organ, the seminal vesicle.

Thus the present experiments tend to confirm those of Deanesly (1938) who grafted ovaries to the ears of rats and concluded that the androgenic activity is associated with the degree of ovarian thecal luteinization. However, Deanesly (1938) and Goodman (1934) and Pfeiffer (1937, transplants of rat ovaries in the anterior eye chamber) are in agreement that such

TABLE 3. STEROID SERIES DATA INDICATING ABSORPTION OCCURS IN THE SEMINAL VESICLE

Group	Animal number	Amount implanted (mg.)	Amount recovered* (mg.)
Estrone	E. 1	3.2	0.45
	E. 2	3.4	0.90
	E. 3	3.9	0.38
	E. 4	4.6	0.00
	E. 5	6.2	4.00
	E. 6	3.5	0.57
	E. 7	5.4	2.10
	E. 8	5.9	0.62
	E. 9	3.3	0.51
	E. 10	4.5	0.70
Progesterone	Pr. 1	2.5	0.0
	Pr. 2	3.5	2.1
	Pr. 3	4.5	1.9
	Pr. 4	5.7	2.4
	Pr. 5	6.1	1.3
	Pr. 6	6.2	2.5
	Pr. 7	6.6	3.2
	Pr. 8	7.6	1.3
	Pr. 9	6.9	4.1
	Pr. 10	9.5	6.5

* Amount recovered was determined colorimetrically.

grafts do not develop corpora lutea in normal or castrated males. The present work describes such developments in the grafts. Lack of formative corpora lutea in grafts placed into the intact male (Goodman, 1934) may be explained on the basis of a limiting luteinizing titer. The absence of such bodies in castrates receiving ovarian transplants may be explained in at least two approaches: 1. the castrated rat's pituitary secretes excessive quantities of follicle stimulating hormone (Du Shane *et al.*, 1935); and 2, the male pituitary normally secretes smaller quantities of luteinizing hormone than does that of the female (Pfeiffer, 1937). On the other hand an end organ—the ovarian graft—is present; consequently it is conceivable that a pituitary-gonad relationship is established in these grafted animals so that production of excessive FSH may be prevented. Moreover, Moore (1919) and Wang *et al.* (1925) have described corpora lutea formation in grafts made to the recti muscles of castrated male rats. It might

therefore be postulated that the abdominal cavity is the normal site for ovarian histogenesis while the eye and ear sites present conditions abnormal to the gland. If this explanation suffices, the ever-present possibility of strain and individual differences in the extent of L.H. elaboration by the male pituitary need not be stressed.

Ovarian implanted vesicles of castrated-adrenalectomized animals were stimulated to the same degree as those possessed by rats which were only castrated. Therefore, it seems apparent that the andromimetic activity of the grafted ovary is not potentiated nor mediated by the adrenal.

No connective tissue hypertrophy was observed in vesicles implanted with testis or testosterone; but such an effect was seen in some ovarian-implanted vesicles and the placental group. The fibrotropic effect was more noticeable in vesicles containing ovarian grafts which were only slightly androgenic. Since estrogens administered intravenously or subcutaneously (Emmens and Parkes, 1947) produce fibrotic vesicles, it was reasoned that these grafts were elaborating estrogenic substance. Because estrone crystals were ineffective in this regard, it is presumed that this substance is not fibrotropic, at least when employed in this manner.

Although androgen has been extracted from human placenta (Cunningham and Kuhn, 1941), the present investigations do not disclose any such activity in rat placenta. Our negative results using 10.9 mg. (mean implant-weight) of placenta are instructive, since such relatively small quantities of tissue may elaborate more secretion in 25 days than can be extracted from the whole organ at any one time. However, it is conceivable that if the order of androgenic elaboration of these grafts is at a threshold below that required for activation of the vesicular epithelium, it would be undetectable. On the other hand, in regard to androgen production, human and rat placentae may not be comparable.

With respect to androgenicity, negative results were also obtained with progesterone crystals. This seems to confirm the experiments of Hill (1937a) and Pfeiffer and Hooker (1942). Although Greene *et al.* (1939, 1940) have pointed out that huge doses of this material are necessary to produce an effect when injected subcutaneously, it seems likely that some stimulating capacity should have been observed in our experiments in view of the fact that the target organ epithelium is in such close proximity to the crystalline material. However, it is possible that the end organ cannot utilize the substance when presented in this manner. Testosterone crystals on the other hand were apparently almost completely absorbed since a residue was recovered in only one instance. This preferential absorption might indicate that either the propionate is in a form more readily utilizable or that the host tissue is more avid in its metabolic requirements for testosterone than for any other steroids investigated.

Autografts and homoiografts of testicular tissue maintained the accessories quite well. Testosterone crystals were most potent in this regard since the vesicle receiving the hormone weighed more than twice that of the normal, while the mate vesicle and ventral prostatic lobes possessed weights which were in the normal range. These observations are borne out by histological examination. Since in each series wherein androgenic effects were observed, the greatest stimulation was seen in close proximity to the graft, the establishment of an androgenic field emanating from the implant is indicated.

SUMMARY

Ovarian material obtained from virgin and pregnant rats of varying ages was transplanted to a seminal vesicle of an adult castrated or castrated-adrenalectomized male rat. This procedure was seen to prevent, to a considerable extent, the regressive changes in the reproductive accessories which are the normal sequelae of castration. The androgenic capacity is reflected in histologically and cytologically evident signs of stimulation as well as partial weight maintenance of the seminal vesicle-prostate complex of the castrated hosts. Implants of 15-day old testis as well as autografts of this material also serve to maintain the accessories, while grafts of pancreatic, salivary, thyroid, fetal membrane and placental tissue do not alter the castrate picture.

Cholesterol, estrone and progesterone crystals introduced into the vesicle have no androgenic effects but the efficacy of testosterone propionate crystals is well demonstrated since each organ receiving this steroid weighs more than twice as much and shows greater histological activity than the normal organ. The contralateral gland and the ventral prostate of these animals are identical with that of the normal animal.

Microscopic examination of the serially sectioned grafts reveals many ovaries to be essentially normal. Correlation of ovarian histology with the greatest degree of androgenicity appears to involve luteinization of the follicles. The testicular grafts assume the typical cryptorchid condition. The pancreatic grafts become necrotic and salivary implants regress but the autografted thyroid as well as the fetal membrane and placental grafts appear well differentiated.

Examination of recovered transplants of corpora lutea reveals that development of apparently full sized ovaries occurs. Proliferation of fragments of ovary adhering to the corpora lutea may be presumed to produce this result.

For his assistance in these analyses and the supply of hormones used in this work, we are indebted to Mr. Herman Cohen of Squibb and Sons, New Brunswick, N. J.

It is concluded that an ovarian graft displays androgenic activity when transplanted to the seminal vesicle and that lower thermal surroundings are, therefore, not indispensable for androgen production by such transplants.

REFERENCES

COHEN, H. AND R. W. BATES: *J. Clin. Endocr.* **7**: 701. 1947.

CUNNINGHAM, B., AND H. H. KUHN: *Proc. Soc. Bio. and Med.* **48**: 314. 1941.

DEANESLY, R.: *Proc. Roy. Soc. London.* **126**(B): 122. 1938.

DORFMAN, R. I.: The Hormones. p. 469, Academic Press, N.Y.C.

DU SHANE, G. P., W. T. LEVINE, C. A. PFEIFFER AND E. WITSCHI: *Proc. Soc. Exper. Bio. and Med.* **33**: 339. 1935.

EMMENS, C. W. AND A. S. PARKES: *Vitamins and Hormones.* **5**: 233. 1947.

GOODMAN, L.: *Anat. Rec.*, **59**: 233. 1934.

GREENE, R. R., M. W. BURRILL, AND A. C. IVY: *Endocrinol.*, **24**: 351. 1939.

GREENE, R. R., M. W. BURRILL, AND D. M. THOMPSON: *Endocrinol.* **27**: 469. 1940.

HASKINS, A. L.: *Proc. Soc. Exper. Bio. and Med.* **70**: 228. 1949.

HILL, R. T., AND W. U. GARDNER: *Anat. Rec.* **64**: S. 21. 1936.

HILL, R. T.: *Endocrinol.* **21**: 495. 1937a.

HILL R. T.: *Endocrinol.* **21**: 633. 1937b.

KATSCH, S., A. S. GORDON AND H. A. CHARIPPER: *Anat. Rec.* **101**: 47. 1948.

MAYER, I.: *Virchow's Arch.* **309**: 625. 1942.

MOORE, C. R.: *J. Exp. Zool.* **28**: 137. 1919.

PFEIFFER, C. A.: *Am. J. Anat.* **58**: 195. 1937.

PFEIFFER, C. A., AND C. W. HOOKER: *Anat. Rec.* **83**: 543. 1942.

WANG, G. H., C. P. RICHTER, AND A. F. GUTTMACHER: *Am. J. Physiol.* **73**: 581. 1925.

NOTES AND COMMENTS

EFFECTS OF PURIFIED ACTH ADDED *IN VITRO* ON THE OXYGEN CONSUMPTION AND ASCORBIC ACID CONTENT OF SURVIVING DOG ADRENAL SLICES[1]

Carpenter, MacLeod and Reiss (1946) have reported an increased consumption of oxygen by rat adrenal cortices 24 hours after the administration of the ACTH *in vivo*. The experiments reported herein were undertaken to ascertain whether or not purified ACTH added *in vitro* has a demonstrable effect on the oxgen consumption of surviving slices of dog adrenal cortex.

EXPERIMENTAL METHODS AND DESIGN

Ten mongrel dogs were used as sources of adrenal slices. These animals had been studied six weeks before in a medical student class experiment in pharmacology involving the estimation of sulfonamide concentrations in blood and spinal fluid samples. Although all of them had been anesthetized at that time with pentobarbital sodium they recovered from the procedure and were apparently in good health at the time they were sacrificed.

The experiments to be reported were done on two days; five dogs were used on each day. The animals were anesthetized with intraperitoneal injections of pentobarbital sodium (approximately 40 mg./kg. of body weight). This was done to avoid a convulsive type of death that may have resulted from other techniques of sacrifice. The adrenals were removed, cleaned, weighed on a torsion balance and stored in a moist, chilled chamber until all the glands had been collected. At that time the V-shaped glands were bisected at the apex of the V and slices were prepared according to the method of Deutsch (1936). The capsule of each flat adrenal segment was removed and a slice of cortex not visibly contaminated with medulla was obtained; the segment was then turned over and the procedure was repeated. In this way, a pair of slices of cortex was obtained from each segment of each gland. The pairs were stored together on physiological saline-moistened filter paper in an ice-chilled chamber until the vessels were loaded. At this time it was possible to identify the paired slices and separate them into pairs of vessels, one of which contained only glucose-phosphate buffer (pH 7.4, Krebs and Henseleit, 1932), and the other, glucose-phosphate buffer plus 5 mg. of purified ACTH* in the side-arm. Not infrequently, more than one homologous pair of slices had to be used to bring the total weight of slices up to a minimum of 50 mg. per vessel. The average sample wet weight per vessel was about 80 mg. After the vessels were loaded they were filled with oxygen, the ACTH was tipped into the main chamber and a 15 minute equilibration period of shaking at 37.8°C. and 112 oscillations per minute was begun. Although zero time for the oxygen-consumption readings was about 2½ hours after the first dog's adrenals had been removed, it should be emphasized that the tissue was stored in moist chilled chambers during most of this interval. Oxygen consumption was measured by the direct method in vessels of approximately 12.5–13.0 ml. volume.

Received for publication June 16, 1950.

[1] Aided by a grant from the American Cancer Society administered by the Committee on Growth of the National Research Council.

* Armour, Lot No. 60-61, kindly supplied by Mr. Irby Bunding of Armour and Co.

The measurements were made over a period of 120 minutes; the rate of oxygen consumption did not appear to diminish during the second hour.

At the end of the experiment, filter paper wicks were removed from the center walls and the alkali carefully blotted with absorbent cotton. Quantitative transfers of the entire contents of each vessel were made by means of repeated washings with metaphosphoric-acetic acid solution and analyses for ascorbic acid were carried out according to the technique of Roe and Keuther (1943).

RESULTS

It will be seen in Table 1 that the mean oxygen consumption of the control adrenal slices was 83.1 S.E. ±4.5, whereas the corresponding figure for the slices to which ACTH was added *in vitro* was 103.7 S.E. ±3.7. The difference between the means is regarded as a significant one on the basis of Fisher's "t" test.

TABLE 1. EFFECT OF PURIFIED ACTH ADDED IN VITRO UPON THE OXYGEN CONSUMPTION OF DOG ADRENAL SLICES

Vessel contents	No. of samples	No. of dogs	Oxygen consumption ($mm.^3$/100 mg. wet wt./hr.)		Probability*
Control**	16	10	83.1	4.5 (S.E.)	<0.01
ACTH, 5 mg.	16	10	103.7	3.7	

* Fisher's table of "t."
** 2.8 ml. of phosphate buffer, pH 7.4, containing 0.2% glucose.

Since the stimulation of the metabolism of the adrenal slices may have been a nonspecific one it was pertinent to inquire whether or not ACTH added *in vitro* produced changes in the chemical morphology of adrenal tissue comparable with those seen following its administration in the intact animal. Therefore, ascorbic acid analyses were made of the total contents of each flask and of a few unincubated slices as well. The results are shown in Table 2. While there is no significant difference between the ascorbic acid content of unincubated slices and those incubated without ACTH, the mean value for the ACTH group was significantly lower than that of either of the other groups.

TABLE 2. EFFECT OF PURIFIED ACTH ADDED IN VITRO UPON THE ASCORBIC ACID CONTENT OF DOG ADRENAL SLICES

Group	Description	No. of samples	No. of dogs	Ascorbic acid mcg./100 mg. wet tissue	Probability*
A	Unincubated slices	7	3	140.6 ±8.2 (S.E.)	AB >0.05
B	Incubated slices	16	10	128.9 ±3.8	BC <0.01
C	Incubated slices plus ACTH	14	9	111.5 ±2.6	AC <0.01

* Fisher's table of "t."

CONCLUSION

When purified ACTH is added *in vitro* to slices of dog adrenal cortex a) the oxygen consumption of the tissue is significantly increased and b) its ascorbic acid content is significantly depressed.

JAY TEPPERMAN, *with the technical assistance of* JANET M. DEWITT
From the Department of Pharmacology
New York State University Medical Center
Syracuse, N. Y.

REFERENCES

CARPENTER, R. K., L. D. MACLEOD, AND M. REISS: *J. Physiol.* **105:** 231. 1946.
DEUTSCH, W.: *J. Physiol.* **87:** 56 P. 1936.
KREBS, H. A., AND K. HENSELEIT: *Z. physiol. Chem.* **210:** 33. 1932.
ROE, J. H., AND C. A. KEUTHER: *J. Biol. Chem.* **147:** 399. 1943.

LACK OF EFFECT OF DESOXYCORTICOSTERONE ACETATE AND ASCORBIC ACID ON FORMALDEHYDE-INDUCED ARTHRITIS IN RATS

Several reports have been published describing symptomatic improvement of rheumatoid arthritis following injection of desoxycorticosterone acetate (DCA) followed immediately by injection of ascorbic acid. Others, however, have not been able to confirm this observation (Bywaters *et al.*, 1950; references to other clinical reports in this paper). Brownlee (1950) studied the influence of DCA plus ascorbic acid on formaldehyde-

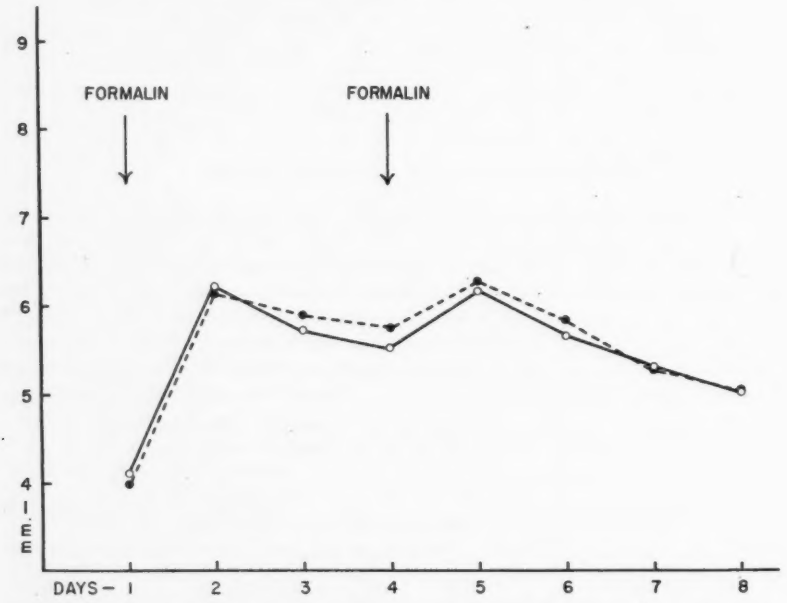


FIG. 1. Effect of DCA (- - -) and DCA+ascorbic acid (——) on formalin-induced arthritis in adrenalectomized rats. Means of measurements of 16 rats treated with DCA and 15 treated with DCA and ascorbic acid.

induced arthritis in rats. He reports that daily subcutaneous injection of 1 mg DCA, followed immediately by intraperitoneal injection of 20 mg of ascorbic acid, protect normal and adrenalectomized rats against formaldehyde-induced arthritis.

This observation appeared to us to be of equal interest for treatment of arthritis and

Received for publication June 16, 1950.

for basic problems of the physiology of adrenal hormones. With the latter aspect in view we planned the use of the method for an extensive study. Prior to embarking on this, it was deemed necessary to repeat Brownlee's experiments.

MATERIAL AND METHODS

The technique as described by Brownlee was followed closely. 31 adult male rats, from the Albino Farm, Redbank, New Jersey, were used. Adrenalectomy was performed under ether anesthesia through the usual dorsal approach. Following operation, and throughout the entire experiment, all rats received 0.9% NaCl as drinking solution. Experiments were started on the fourth day after adrenalectomy; this day is hereafter referred to as day one.

Animals were divided into 2 groups, one (16 animals) receiving 1 mg DCA daily by subcutaneous injection, the other (15) animals receiving 1 mg DCA subcutaneously, immediately followed by intraperitoneal injection of 20 mg ascorbic acid.

0.1 ml 2% formalin was injected beneath the plantar aponeurosis of both feet on days one and four of the experiment. Measurements of the a-p diameter of the foot were made daily to the closest millimeter by means of a caliper.

RESULTS

Results are summarized in figure one. There was no difference in size of the tissue reaction to formalin in the animals treated with DCA alone as compared with those treated with DCA and ascorbic acid.

SUMMARY

We have not been able to confirm Brownlee's work. Duplicating his procedure closely we have found no difference of the formaldehyde induced arthritis in adrenalectomized control animals injected with DCA, and those treated with DCA and ascorbic acid. These negative results are reported briefly because of the inportant implications inherent in the problem.

SYLVESTRE FRENK,[1] SIDNEY WOLFE AND KARL E. PASCHKIS
Division of Endocrine and Cancer Research
Jefferson Medical College
Philadelphia, Pa.

REFERENCES

BROWNLEE, G.: *Lancet* **1**: 157. 1950.
BYWATERS, E. G. L., A. ST. J. DIXON, AND J. B. WILD: *Lancet* **1**: 951. 1950.

THE MECHANISM OF INSULIN-INDUCED EOSINOPENIA IN RATS

Long and Fry (1945) demonstrated that epinephrine stimulates the pituitary-adrenocortical axis. This was followed by the reports of Forsham and his associates that ACTH (1948) and epinephrine (1948) induce a fall in the number of circulating eosinophiles which is dependent upon the presence of a functional adrenal cortex. Godlowski (1948) and Laragh and Almy (1948) reported that the number of circulating eosinophiles was depressed in patients following the injection of insulin. Dury (1950) showed that insulin

[1] Present address: Juan Esentia 76, Mexico D. F.

Received for publication June 2, 1950.

and epinephrine were equally effective in inducing an eosinopenia in intact rats which was associated with a depletion of the adrenal ascorbic acid. It is the purpose of this paper to show that the adrenal medulla is essential if an eosinopenia is to be induced after insulin injection.

MATERIALS AND METHODS

Normal intact and adrenal enucleated male Wistar rats weighing 200–250 grams were used. The medullectomized rats were 16–17 days after surgery at the time of the experimental procedures described. They were maintained on the regular chow biscuit diet and 1% NaCl drinking solution, and greens once weekly. Greep and Deane (1949) showed that the adrenal cortices of medullectomized rats were histologically regenerated after two weeks. The adrenal glands of the medullectomized rats used in this investigation were removed at the time of sacrifice for histological study.[1] The adrenal cortices were found to be histologically normal appearing and fully regenerated. Histo-chemical tests showed the presence of reducing (ascorbic acid) and lipid-staining material. These findings will be reported in detail in another paper. There was no histological evidence of adrenal medullary tissue in the medullectomized rats reported in this investigation. Epinephrine was injected subcutaneously at a dosage level of 0.02 or 0.04 mg./100 gm. of rat. The insulin used was prepared fresh for use by diluting Lilly's ILLETIN with physiological saline. This was injected subcutaneously at a dosage level of 0.5 μ (0.6 ml.) per rat. The number of circulating eosinophiles per c. mm. of blood *before* and 180 minutes *after* the injection of epinephrine or insulin was enumerated on tail blood by the direct counting method of Randolph (1944). The Mean-Difference of the before and after eosinophile counts of the rats in each group was calculated and tested for statistical significance. The blood glucose level of the rats at the end of the 180 minutes after the injection of either agent was determined by the method of Somogyi-Schaffer-Hartmann on blood obtained by cardiac puncture. All procedures were carried out on rats which had been fasted overnight (approx. 16 hrs.) and while they were under EVIPAL anesthesia.

The period of 180 minutes after the injection of epinephrine or insulin was chosen on the basis of data in the literature. Forsham and his colleagues have shown that the number of eosinophiles were maximally depressed in patients with adequate adrenocortical function four hours after the injection of ACTH or epinephrine. The adrenal ascorbic acid concentration has been shown to be maximally depleted approximately three hours after epinephrine injection in rats (Long, 1947). Dury (1950) showed that the association of significant depletion of adrenal ascorbic acid and induced eosinopenia occurred in rats 180 minutes after insulin injection but not sooner (30, 60 or 120 minutes).

RESULTS

The change in the number of circulating eosinophiles per c. mm. of blood in normal and adrenal enucleated groups of rats 180 minutes after the injection of epinephrine or insulin is summarized in Table 1. Inspection of these results show that epinephrine and insulin induced an eosinopenia in intact groups of rats. However, in the medullectomized groups of rats epinephrine but not insulin was capable of inducing a significant eosinopenia. That the insulin was physiologically active in the medullectomized rats is shown by the hypoglycemic changes which followed its injection. It is also apparent that the

[1] Dr. Leo Moss, Pathologist, Bradford Hospital, kindly studied the histological sections of the adrenal glands.

TABLE 1. CHANGE IN THE NUMBER OF CIRCULATING EOSINOPHILES AFTER EPINEPHRINE AND INSULIN IN NORMAL INTACT AND ADRENAL ENUCLEATED RATS

Experimental condition	Eosinophiles/c.mm. of blood		Plasma glucose (mg. %)
	Mean-diff ±S.E.	Value of "P"	Mean ±S.E.
Normal intact: Controls (12) (physiol. sal., 0.5 ml.)	+30 ±23	0.23	177 ± 7.6
Epinephrine, s.c. (5) 0.02 mg./100 gm., rat	−93 ±24	0.01	223 ±17.4
Insulin, s.c. (6) 0.5μ, 0.6 ml.	−57 ±18	0.02	96 ± 5.8
Medullectomized: Epinephrine, s.c. (7) 0.04 mg./1 gm., rat	−52 ±15	0.01	337 ±13.7
Insulin, s.c. (7)* 0.5μ, 0.6 ml.	+29 ±49	0.65	173 ± 7.0
Insulin, s.c. (4) 0.5μ, 0.6 ml.	+36 ±23	0.20	104 ±13.5

Numbers in parentheses are the numbers of rats in each group.
* The rats in all the groups except this one had been fasted overnight (approx., 16 hrs.).

fasting or non-fasting status of the insulin-injected rats did not influence the eosinophile response.

DISCUSSION

It is apparent from the data that the adrenal medulla is essential if an eosinopenia is to be induced in rats after the injection of insulin. It is suggested that the mechanism of insulin-induced eosinopenia is not the result of insulin, per se, but of the consequent hypoglycemia as follows: the insulin-induced hypoglycemia results in an increased production of endogenous epinephrine. This epinephrine titre is sufficient to stimulate the pituitary-adrenocortical axis and the release of cortical secretion which is effective in inducing an eosinopenia. The fall in the number of circulating eosinophiles after insulin is therefore an epinephrine effect, in actuality, which is set in motion by the hypoglycemic status of the blood. This mechanism is in agreement with the findings of Gershberg and Long (1948) that insulin depleted the adrenal ascorbic acid of intact rats.

SUMMARY

The eosinopenic effect which follows the injection of insulin in intact rats has been shown to depend upon the presence of the adrenal medulla. It has therefore been suggested that this change in the number of circulating eosinophiles is not an insulin effect but the consequence of an increased liberation of endogenous epinephrine. The probable mechanism is discussed.

ABRAHAM DURY

From the Dorn Laboratory for Medical Research
Bradford Hospital
Bradford, Pa.

REFERENCES

DURY, A.: *Am. J. Physiol.* 163: 96. 1950.

FORSHAM, P. H., G. W. THORN, F. T. G. PRUNTY, AND A. G. HILLS: *J. Clin. Endocrinol.* **8:** 15. 1948.

GERSHBERG, H., AND C. N. H. LONG: *J. Clin. Endocrinol.* **8:** 587. 1948.

GODLOWSKI, Z. Z.: *Brit. Med. J.* **1:** 46 1948.

GREEP, R. O., AND H. W. DEANE: *Endocrinol.* **45:** 42. 1949.

LARAGH, J. H., AND T. P. ALMY: *Proc. Soc. Exper. Biol. & Med.* **69:** 499. 1948.

LONG, C. N. H.: *Federation Proc.* **6:** 461. 1947.

LONG, C. N_4 H., AND E. G. FRY: *Proc. Soc. Exper. Biol. & Med.* **59:** 67. 1945.

RANDOLPH, T. G.: *J. Allergy* **15:** 89. 1944.

RECANT, L., FORSHAM, P. H., AND G. W. THORN: *J. Clin. Endocrinol.* **8:** 589. 1948.

BOOK REVIEW

THE DIAGNOSIS AND TREATMENT OF ENDOCRINE DISORDERS IN CHILDHOOD AND ADOLESCENCE. By Lawson Wilkins, Associate Professor of Pediatrics, The Johns Hopkins University, Baltimore. Charles C Thomas, Publisher, Springfield, Illinois. xx+408 pp., 411 illustrations, 6 in color. $13.00.

This is a timely and valuable book. It is written by a clinician for clinicians. In terse readable prose the author describes the recognizable endocrinopathies and discusses their differential diagnosis and therapeutic indications. He particularly stresses the need for recognition of the true endocrine deficiencies in contrast to the cases of retarded development which need no treatment. He also presents, sometimes in considerable detail, the diagnostic methods which permit the recognition of the more recondite problems.

The book is particularly worthy in that it provides a timely antidote to the flood of endocrine advertisements calculated to engender guilt in any physician who does not prescribe hormone preparations at every opportunity. Moreover, good books on endocrinology being few and far between, it provides a needed source of information for clinicians who, in want of any other, have perforce had to depend upon the brochures of the drug houses. It calls firmly and authoritatively for careful evaluation of each case before therapy is instituted. One can hope it will decrease the hasty and sometimes harmful administration of the ever more potent products which can cause disease as well as cure it.

The book makes no pretense of presenting a thorough account of the scientific experiments which comprise modern endocrinology. For this, the reader is referred elsewhere. Such an attempt to compress the fundamental scientific background, the author fears, leaves him open to the charge of over-simplification and of dogmatism. Fortunately, two facts refute this charge. The first is Dr. Wilkins' obvious mastery of his field, the second is the atmosphere of authenticity created by his presenting mostly first-hand material. For he has called upon literature for cases only in rare and improbable endocrinopathies. The great majority of his illustrations are derived from patients seen at the Harriet Lane Home, The Johns Hopkins Hospital.

One of the book's virtues is the large number of photographs, charts, case histories and other illustrative material. Not only is the typical, or extreme case illustrated, but the range of severity or of complexity is indicated.

The carping critic perhaps could find minor cause for objection. A picture of a single girl appears to represent a normal 18 year old a total of 9 times. Surely, for the sake of variety, more than one normal 18 year old has been seen at Johns Hopkins! Some of the charts, especially in the early chapters, do seem excessively simplified. The term "isosexual" is etymologically unsound and does not feel good in the mouth. But these are minor points, and the book's virtues far outweigh them.

E.W.D.